JOURNAL OF DAIRY SCIENCE

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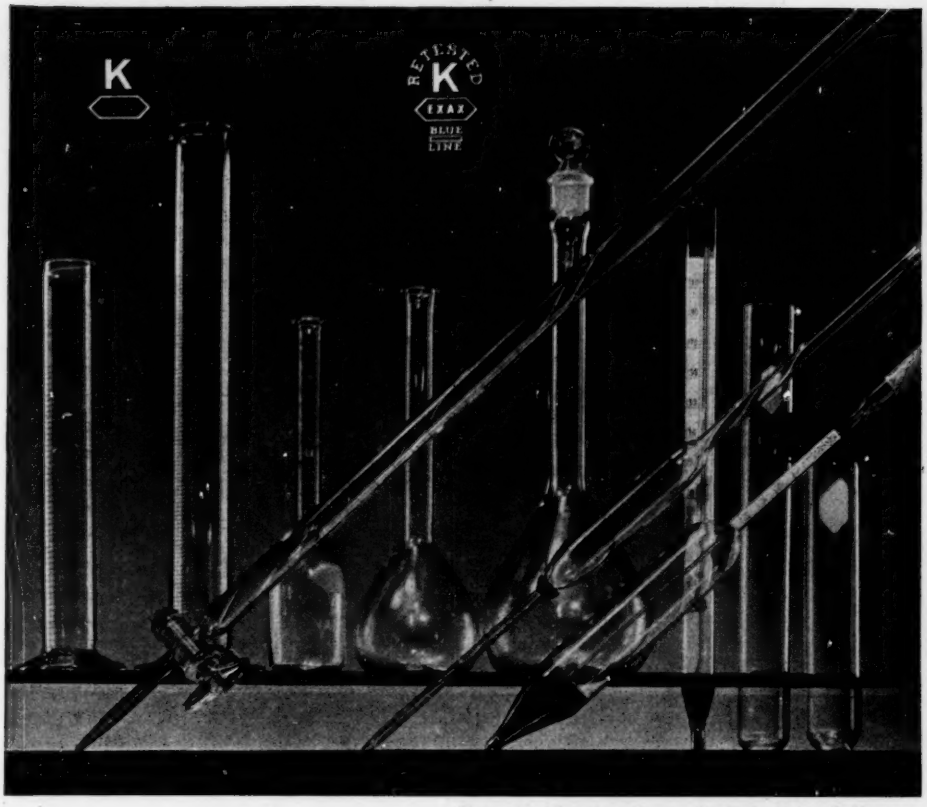

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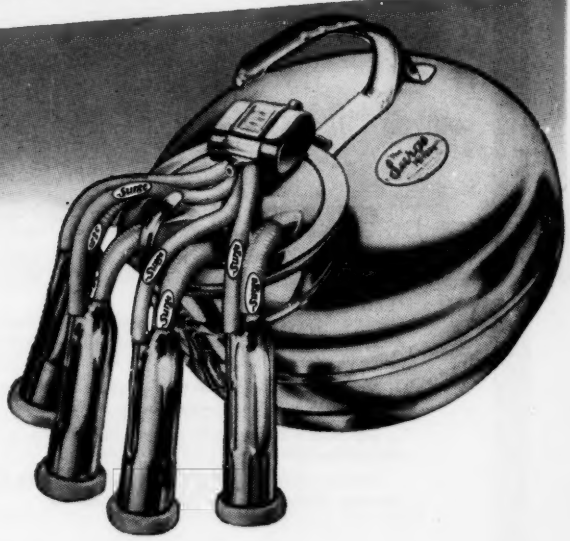

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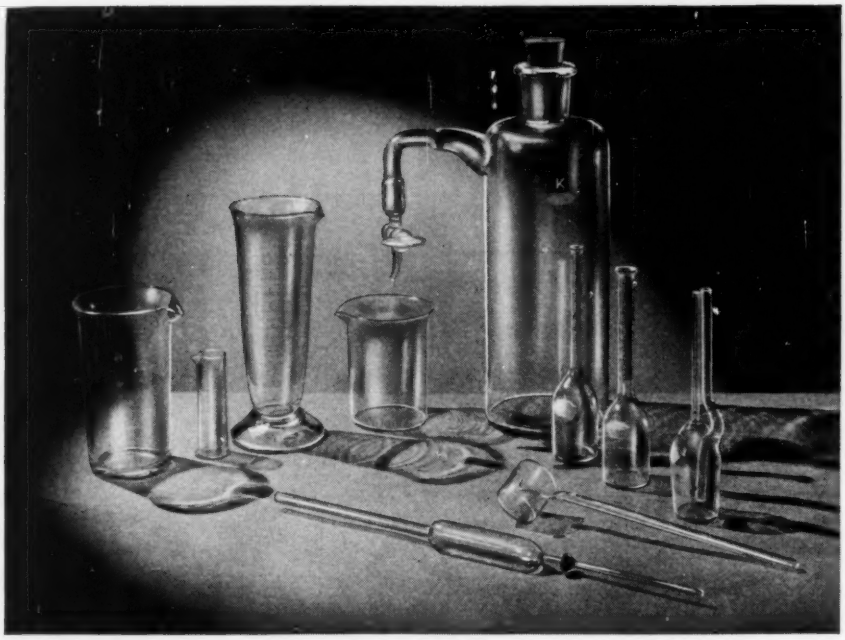

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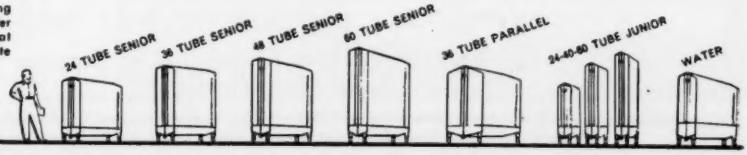

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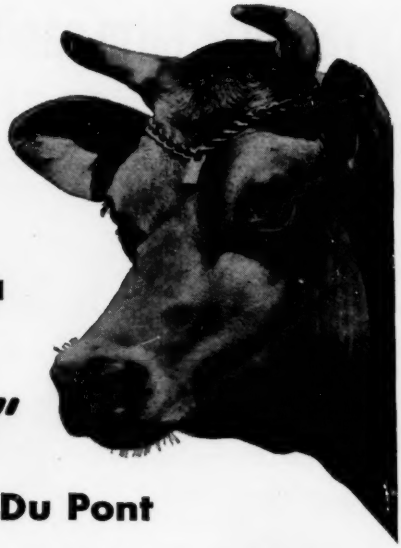

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THE GAS REQUIREMENTS OF MOLDS. V. THE MINIMUM OXYGEN REQIREMENTS FOR NORMAL GROWTH AND FOR GERMINATION OF SIX MOLD CULTURES[1, 2]

D. D. MILLER[3] AND N. S. GOLDING

Division of Dairy Husbandry, Agriculture Experiment Station, State College of Washington, Pullman

Most people assume that molds require abundant oxygen for growth, because molds usually are seen growing on the moist surfaces of substances. This, in a very general way, is correct but establishes no idea of the amount of oxygen required for normal growth or the amount required for germination of the many species of molds. The effect of the temperature and solubility of oxygen is even less understood.

The purpose of this study was to determine the minimum oxygen required for normal growth and the minimum oxygen required to permit germination of six pure cultures of mold in the presence of nitrogen and the almost complete absence of carbon dioxide. Since both temperature and gas pressure change the solubility of oxygen in the medium, several variations in both of these factors were included.

LITERATURE

The literature pertaining to much of the work on the gas requirements of molds has been reviewed by Golding (7, 8). The available literature on the oxygen requirements of molds is limited, with many research workers assuming that abundant oxygen is required for normal growth (5, 6, 11, 12). However, there is available work which shows that various species of molds can grow in very limited supplies of oxygen (1, 3, 16, 17, 18, 19). In several cases molds have given good growth in oxygen concentrations of less than 1 per cent (2, 4, 13, 15).

In a previous paper, Golding (9) has shown that the effect of carbon dioxide on the growth of various molds must be based on the solubility of carbon dioxide in the medium or mycelium. He also has shown that possibly a similar interpretation may have to be given to the effect of oxygen concentrations on molds.

Received for publication July 23, 1948.

[1] Published as Scientific Paper no. 784, College of Agriculture and Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman.

[2] American Dairy Association Research Grant and in cooperation with the Washington State Dairy Products Commission.

[3] The experimental data in this paper are taken from a thesis submitted by the senior author in partial fulfillment for the degree of Master of Science in Agriculture, 1948.

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MATERIALS AND METHODS

Cultures. The following cultures from previous studies (8, 9) were used: *Aspergillus flavus* d, *Aspergillus niger* 14, *Penicillium expansum* 69, *Oospora lactis* m, and *Penicillium roqueforti* 33. *Penicillium notatum* NRRL[4] 832 also was used. This latter mold was purified by the usual pour plate technique (8),

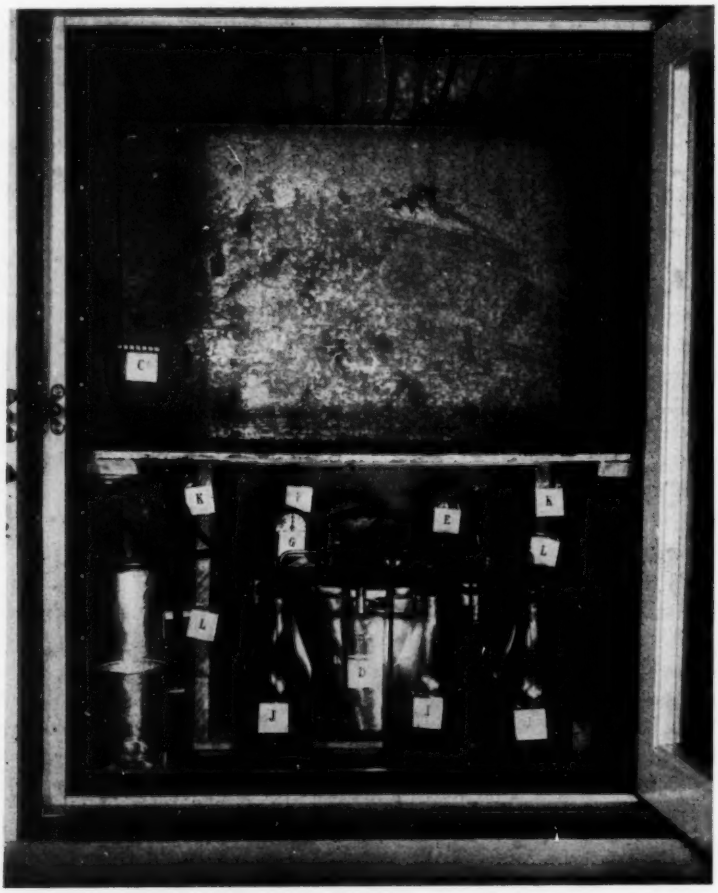

FIG. 1. The internal apparatus in each incubator.

using Difco malt agar as the medium. These species were chosen because of their wide difference in physical characteristics and temperature requirements, and because of their economic importance in food spoilage and in various manufacturing processes.

The same batch of Difco malt agar was used throughout for all plates and

[4] Northern Regional Research Laboratory.

slants used in all experiments. The cultures of mold for inoculation of the plates were grown on malt agar slants at approximately 75° F. for 10 days. The preparation and inoculation of the plates were identical with the previous studies (7, 8, 9). After inoculation, which required approximately 1 hour, the plates were inverted, placed in their respective incubation chambers and incubated for a 7 day period.

Iucubation. The same battery of incubators was used as in the previous studies (7, 8, 9). Figure 1 shows the internal apparatus in each incubator. The temperature of each incubator was controlled by a thermostat *C*. To allow either a vacuum or a pressure under which to grow the molds in the incubation chamber, 8-quart (All-American Queen) pressure cookers *D* were used, each being fitted with an inlet *E* and an outlet *F* which allowed pressure rubber

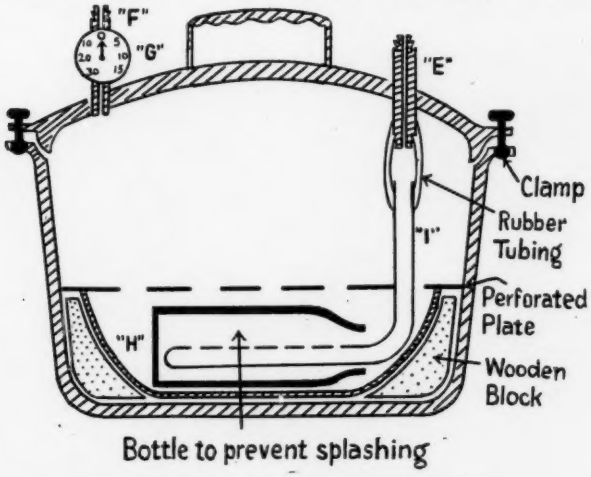

FIG. 2. A cross-section drawing of each cooker.

tubing connection. A pressure-vacuum gauge *G* was fitted to the outlet connection *F*. Figure 2 shows a cross-section drawing of each cooker *D*. In the bottom of each cooker *D*, a pyrex bowl *H* was placed containing saturated ammonium sulfate solution which controlled the humidity inside the cooker *D* at approximately 80 per cent. A similar method of humidifying has been used in previous studies (8). A perforated glass tubing *I* was so constructed as to be attached to the inlet *E* by rubber tubing, and thereby allowed the gas to bubble through the saturated ammonium sulfate solution before being a part of the gas supply of the cooker *D*.

Preceding the inlet *E*, a series of bubbling bottles *J* were placed and the gas used was bubbled continuously through 5 per cent sodium hydroxide solution to remove the carbon dioxide. Thus, the gas used contained a mixture of oxygen and nitrogen at a constant composition during the 7-day incubation period. Gas analysis established this composition.

Two tested thermometers *K* were placed one on either side of the cooker *D* and read and recorded each morning and evening. The average reading of these thermometers was considered as the temperature of incubation for the period. Each cooker was surrounded by a wooden box *L* to shield the cooker from the heating source. The slight variations in temperature from day to day seldom exceeded 2° F.

Control of gas supply. Figures 3 and 4 show the arrangement which allowed a continuous circulating system of gas supply by the use of a gas reservoir *M* and a Kelvinator refrigerator pump *N*. The gas reservoir was a 24 cubic foot cylindrical tank in which the desired oxygen concentration was ob-

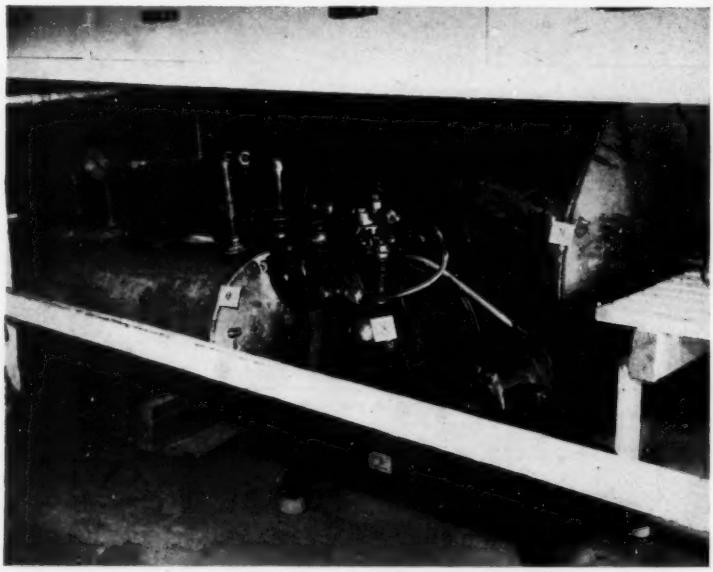

FIG. 3. Gas storage and circulation equipment.

tained by the use of air and dry nitrogen. A pressure of at least one atmosphere above the desired pressure in the cookers was maintained in this reservoir. By the use of a reducing valve *O*, the desired pressure in the cookers was regulated. Seamless copper tubing *P* carried the gas from the reservoir to the cookers and back through the refrigerator pump into the reservoir. An oil trap *Q* preceded the reservoir. This system gave a continuous flow of a desired concentration of gas over the inoculated petri dishes. To maintain the same gas concentration inside the petri dishes as was in the remainder of the cooker, it was found necessary to vary the pressure inside the cookers. This was accomplished by placing a roller *R* on a cam and gearing down so that the cam rotated once each 40 seconds. The roller cut off the gas supply by pressure on the rubber tubing which connected the reducing valve to the seamless

copper tubing. The method caused a fluctuation ranging from 10 to 20 mm. pressure above the plates each 40 seconds. Preliminary tests proved that such a condition gave more uniform growth between the same culture in different petri dishes under like conditions.

Gas analysis. The gas flowing through the system was analyzed every other day during the growth period. A Burrell-Haldane cabinet model gas analysis apparatus was used. The average of all analyses during the growth period was taken as the concentration of gases for that experiment.

Measurement of colony. The measurement of colony was identical with a previous study (8).

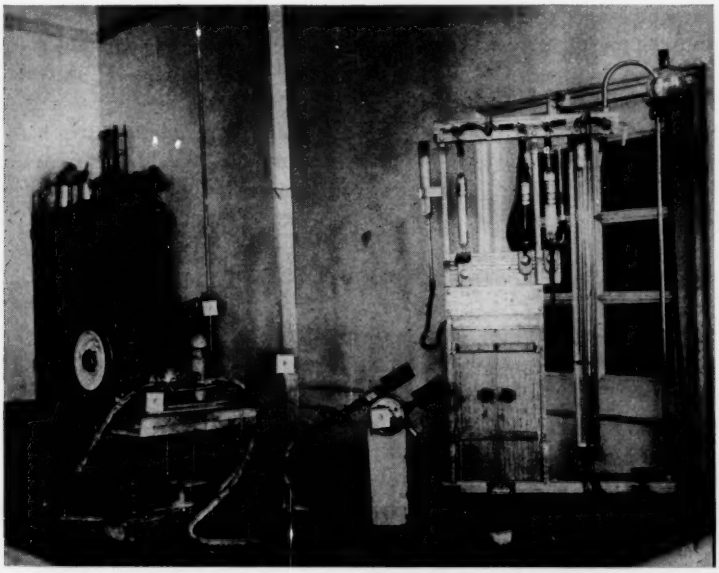

FIG. 4. Gas pressure control apparatus.

Expression of growth. Graphs were obtained by plotting the millimeters of growth as the ordinate and the volume of oxygen at normal temperature and pressure soluble in 1000 volumes of water as the abscissa (fig. 5). The millimeters of growth were obtained by interpolation from growth curves (14) similar to those used in previous studies (8, 9). It was assumed that the volume of oxygen soluble in the culture medium was the same as its solubility in water.

The volumes of oxygen at normal temperature and pressure soluble in 1000 volumes of water were obtained by the following formula:

$$\frac{\text{mm. pressure less vapor pressure}}{760} \times \frac{\text{partial pressure of oxygen}}{100} \times \text{oxygen absorption coefficient (10)} \times 1000 = \text{volumes oxygen at N.T.P. soluble in 1000 volumes of water}$$

Example: *Oospora lactis* grown at 75° F. and 0.312 per cent oxygen showed by interpolation from growth curves a diameter of 48 mm. The average pressure during growth was 694.2 mm. mercury.

$$\text{Thus: } \frac{694.2 - 22.3}{760} \times \frac{0.312}{100} \times 0.02886 \times 1000 = 0.080 \text{ volumes oxygen at N.T.P. soluble in 1000 volumes of water}$$

EXPERIMENTAL

Figure 5 shows the effect of dissolved oxygen in water on the growth of six species of molds at various pressures. The figure shows that there is no significant difference in rate of growth between approximately 0.5, 1 and 2 atmospheres pressure, and that the effect of inhibition of growth is dependent upon the volume of oxygen dissolved in water. In air the volume at normal temperature and pressure of oxygen soluble in 1000 volumes of water ranged from 22.1 at 0.5 atmosphere and 85° F. to 132.9 at 2 atmospheres and 55° F. All six molds reached their normal growth in air at all temperatures before the volume of oxygen at normal temperature and pressure reached one part soluble in 1000 volumes of water. Therefore, for simplicity the curves are not extended beyond that concentration in this paper, but are completed in the thesis (14).

The optimum growth of the six cultures of mold were as follows: *O. lactis*, 85° F.; *P. roqueforti*, 77° F.; *A. flavus*, 90° F.; *A. niger*, 93° F.; *P. expansum*, 76° F.; *P. notatum*, 75° F. Since each culture had approximately the same break in the curve at all growing temperatures at a given oxygen solubility (a function of temperature and pressure), only the 75° F. growth curve for each is given.

O. lactis was not inhibited significantly until the oxygen supply was below 0.3 volumes soluble in 1000 volumes of water. This culture still showed growth in 7 days when the volume of oxygen soluble in 1000 volumes of water was too small to measure by analysis.

P. roqueforti was inhibited significantly when the dissolved oxygen reached approximately 0.78 volumes of oxygen soluble in 1000 volumes of water. No germination in 7 days was recorded at approximately 0.08 volumes of oxygen soluble in 1000 volumes of water.

A. flavus was inhibited significantly at approximately 0.38 volumes of oxygen soluble in 1000 volumes of water. No germination in 7 days was obtained when 0 volumes of oxygen soluble in 1000 volumes of water were used.

A. niger was inhibited significantly at approximately 0.56 volumes of oxygen soluble in 1000 volumes of water. No germination in 7 days was obtained at 0.01 volumes of oxygen soluble in 1000 volumes of water.

P. expansum was inhibited significantly at approximately 0.56 volumes of oxygen soluble in 1000 volumes of water. No germination in 7 days was obtained at approximately 0.04 volumes of oxygen soluble in 1000 volumes of water.

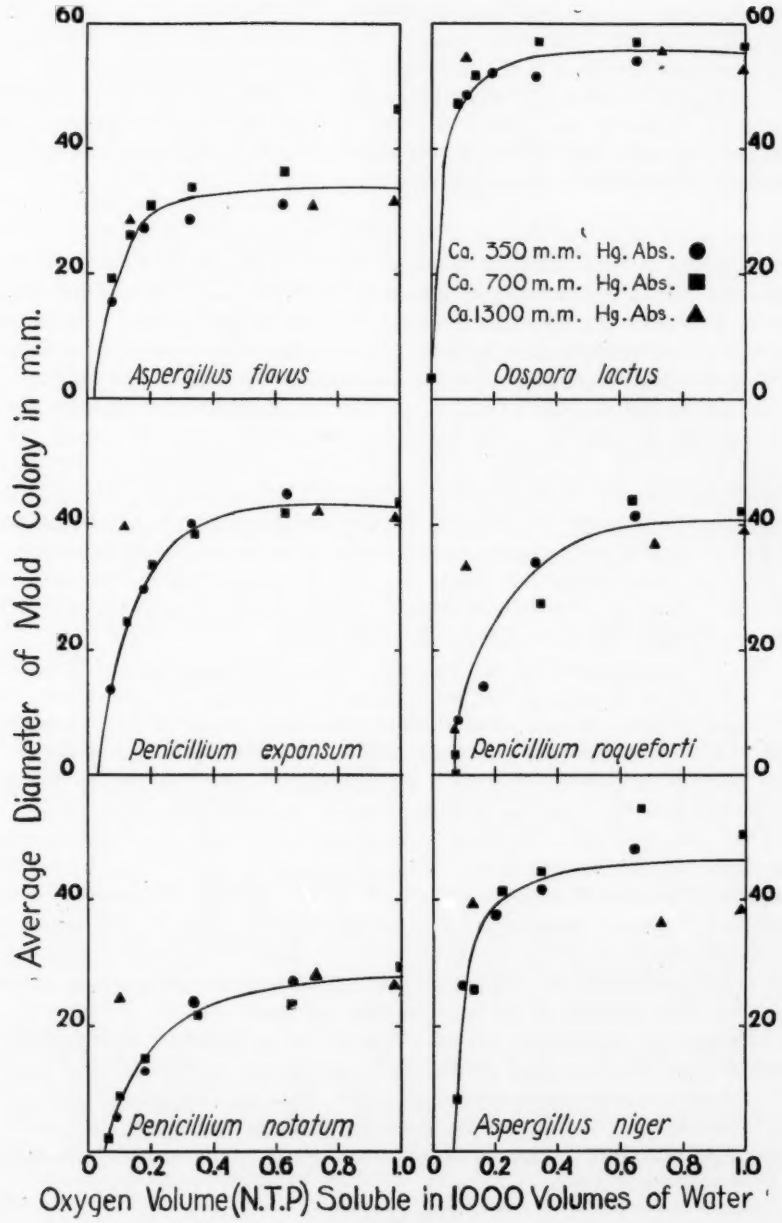

FIG. 5. The effect of dissolved oxygen on the growth of molds at 75° F.

P. notatum was inhibited significantly at approximately 0.8 volumes of oxygen soluble in 1000 volumes of water. No germination in 7 days was obtained at approximately 0.06 volumes of oxygen soluble in 1000 volumes of water.

The various curves show that the amount of dissolved oxygen required for inhibition of growth varies from *O. lactis* as the lowest to *P. roqueforti* as the highest. The sequence of the six cultures was *O. lactis, A. flavus, A. niger, P. expansum, P. notatum* and *P. roqueforti.*

DISCUSSION

From the data presented, it is shown that when the growth of molds is not restricted due to lack of oxygen, pressures of 0.5, 1 and 2 atmospheres do not significantly influence growth. It is seen that the oxygen supply must be quite low before any restriction of growth of the molds takes place. When this reduction occurs, the oxygen requirement of molds is dependent upon the dissolved gas in the medium or mycelium and not directly with the composition of oxygen in the gas above the medium. Where restriction of growth occurs due to low oxygen, the supply of this gas to the mold is influenced by the absorption coefficient and Henry's law. These data verify the previous study (9),

Workers who study the effect of oxygen and possibly other gases on mold growth at various temperatures and pressures must convert their data to terms of dissolved gases rather than using the data on the gas composition above the medium. Otherwise, the results obtained will be useful only where exactly the same temperature and atmospheric conditions exist.

The amount of dissolved oxygen used in this experiment ranged from 132.9 to 0 volumes of oxygen at normal temperature and pressure soluble in 1000 volumes of water. No appreciable effect was noted on any of the six cultures used until the volume of oxygen was below 0.8 volumes of oxygen soluble in 1000 volumes of water. Therefore, the amount of dissolved oxygen required for normal growth of various species of molds is exceedingly low.

There is a noticeable difference in the oxygen requirements of different species of molds. Of the six cultures used in this experiment, *P. roqueforti* showed the greatest amount of inhibition of growth at low volumes of dissolved oxygen. Inhibition of growth began at 0.8 volumes of oxygen soluble in 1000 volumes of water. *O. lactis,* on the other hand, did not show appreciable inhibition of growth until 0.3 volumes of oxygen soluble in 1000 volumes of water was reached. The other four cultures ranged between these extremes.

The amount of dissolved oxygen required for germination at temperatures which give growth in a 7 day period was extremely low for the cultures studied. *O. lactis* showed appreciable growth when the dissolved oxygen content was too low to measure by the Burrell-Haldane gas analysis apparatus. All cultures germinated at a dissolved oxygen content above 0.08 volumes of oxygen soluble in 1000 volumes of water.

CONCLUSIONS

1. The oxygen requirements of *O. lactis, P. roqueforti, A. flavus, A niger, P. expansum* and *P. notatum* were studied at various percentages of oxygen

above the medium at various temperatures and pressures. It was found that the inhibiting effect of reduced oxygen is in proportion to its solubility and not directly in proportion to the composition of the gas above the medium or mycelium. Pressures between 0.5 and 2 atmospheres in air do not affect the oxygen requirements of the cultures studied.

2. No inhibitory effect on any culture was noted until the dissolved oxygen was below 0.8 volumes of oxygen at normal temperature and pressure soluble in 1000 volumes of water.

3. Complete stoppage of germination of any culture for 7 days was not obtained until the dissolved oxygen content was below 0.08 volumes of oxygen at normal temperature and pressure soluble in 1000 volumes of water. *O. lactis* showed appreciable growth when no measurable oxygen was present.

4. There is a significant difference in the oxygen requirements of different species of molds. Of the six cultures studied, *O. lactis* required the least oxygen and *P. roqueforti* the most.

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DETERMINATION OF NITROGEN IN MILK BY DIRECT NESSLERIZATION OF THE DIGESTED SAMPLE

J. H. HETRICK AND R. M. WHITNEY[1]

Dean Milk Company, Rockford, Illinois

Interest in the changes in the protein fractions present in milk due to both season and treatment made it necessary to develop a rapid method for the determination of nitrogen. The macro and even the semi-micro methods presented in the literature were not sufficiently rapid to allow for a large enough number of determinations to yield the information desired.

HISTORICAL

Since the inception of the Kjeldahl (7) method for the determination of organic nitrogen, a large amount of research has been done, broadening its application and improving its technique. It has been applied on a macro, semi-micro and micro scale in innumerable instances. An excellent review and bibliography of the work has been supplied by Bradstreet (3). This method long has been used in the determination of nitrogen in dairy products and is employed as the official method of the Association of Official Agricultural Chemists (2). As outlined in the macro scale, it is time-consuming, especially when fractionation of the organic nitrogen components of milk is desired. Rowland (10) and Menefee and Overman (9), in recognizing this, have developed semi-micro methods which have reduced considerably the time required for a determination. However, even these methods require fairly long digestion time and additional time for distillation and titration of the ammonia formed. When small amounts of ammonia are present in the sample or when micro samples are used, advantage has been taken of the Nessler reaction in place of titration. In the majority of cases (5), the Nessler reagent was added to the distillate or to a solution of ammonia obtained by aspirating air through the digested solution and into distilled water, in order to eliminate interferences present in the sample. However, in the cases of blood and urine (8), direct nesslerization of the digested mixture has been employed. Kieferle and Gloetzl (6) applied direct nesslerization to the serums obtained in the study of the action of a large number of precipitants upon the milk proteins. The work presented in this paper consists of a further investigation and modification of their technique and its application to the study of the organic nitrogen constituents of milk.

METHOD

Reagents:

(a) NH_3-free water: Prepare by redistilling distilled water in a 2-l. all-Pyrex glass still in the presence of 10 ml. of concentrated H_2SO_4. The distillate

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[1] Present address: Department of Food Technology, University of Illinois, Urbana.

should be tested according to the following procedure and discarded if it contains any NH_3, as indicated by the test.

(b) K_2SO_4, low nitrogen content.

(c) H_2SO_4, C. P., low nitrogen content.

(d) H_2O_2, 30 per cent.

(e) Gum acacia solution 2 per cent w/v: Dissolve 2 g. of good-quality powdered gum acacia in NH_3-free water in a 100 ml. volumetric flask and make up to volume. Add 1 ml. of CCl_4 as a preservative. This solution should be clear and should be discarded when turbidity develops.

(f) Nessler's Reagent: Dissolve 50 g. of KI, C.P., in 35 ml. of cold NH_3-free water and add a saturated solution of $HgCl_2$, C.P., until the orange precipitate formed persists. Add 400 ml. of an approximately 9 *N* NaOH solution (360 g. of NaOH, C.P., in 1 l. of NH_3-free water) and dilute the resultant solution to 1 l. with NH_3-free water. This solution should be allowed to stand for several days before use, in order for equilibrium to be established.

(g) Standard NH_4Cl stock solution (1 ml. $\simeq$ 1 mg. N): Dissolve 1.9090 g. of NH_4Cl, C.P., in NH_3-free water and dilute to volume in a 500 ml. volumetric flask. Before using, dilute this solution further with NH_3-free water so that 1 ml. $\simeq$ 0.01 mg. N.

(h) $K_2Cr_2O_7$ solution 1 per cent w/v: Dissolve 1 g. of $K_2Cr_2O_7$, C.P., in distilled water and make up to 100 ml.

Apparatus:

(a) Weight pipettes with weighing cross, if liquids are used.

(b) Analytical balance and weights.

(c) Micro-Kjeldahl digestion rack.

(d) Micro-Kjeldahl flasks, calibrated to contain 50 ml., if it is desired.

(e) Glass beads, washed with concentrated H_2SO_4 and thoroughly rinsed with NH_3-free water.

(f) Small measuring spoon, to contain approximately 0.4 g. of K_2SO_4.

(g) Spectrophotometer with accessories (Coleman-Model 11).

(h) Standard calibrated glassware.

Permanent color blank. Transfer a measuring spoonful of K_2SO_4, 0.5 ml. of concentrated H_2SO_4, one glass bead and 12.5 ml. of NH_3-free water to each of five micro-Kjeldahl flasks and digest on the rack for exactly 3 minutes after the appearance of SO_3 fumes. Cool and add three drops of 30 per cent H_2O_2, being sure that it falls directly into the liquid in each flask. Digest for exactly 3 minutes more, cool to room temperature, dilute with NH_3-free water, add 1 ml. of 2 per cent gum acacia solution and make up to 50 ml. with NH_3-free water. Transfer 10 ml. aliquots to test tubes and place the tubes in a thermostat at a convenient temperature $\pm$ 1.0° C. (in this study the temperature selected was 23° C.). Add exactly 2 ml. of Nessler's reagent to each tube and measure the percentage transmission on the spectrophotometer at 420 mμ 10 minutes after the addition of the reagent, using distilled water as a blank. These values should not deviate from each other by more than the instrumental error.

To a series of test tubes containing 10 ml. of distilled water, add 0.1, 0.2, 0.3 and 0.5 ml. of 1 per cent $K_2Cr_2O_7$ solution and measure the percentage transmission at 420 mμ on the spectrophotometer, using distilled water as a blank. Plot the percentage transmission against the concentration of $K_2Cr_2O_7$ on semi-log graph paper, and from the curve obtained, determine the concentration of $K_2Cr_2O_7$ necessary to yield the same percentage transmission as the average of the reagent blanks. Figure 1 illustrates this process. Make up a $K_2Cr_2O_7$ solution of this concentration to use as a permanent color blank. The required strength of this solution will vary with the nitrogen content of the reagents used. For

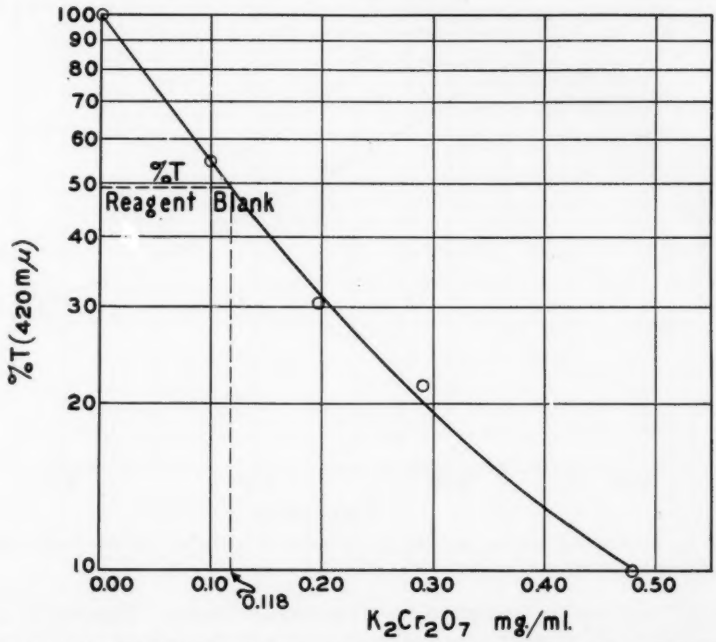

FIG. 1. Relationship between percentage transmission at 420 mμ and concentration of $K_2Cr_2O_7$ solution.

comparison purposes, the solution used in this study consisted of 6.0 ml. of 1 per cent $K_2Cr_2O_7$ diluted to 500 ml.

Standard curve of percentage transmission vs. nitrogen concentration. Transfer a measuring spoonful ($\sim$0.4 g.) of K_2SO_4, 0.5 ml. of concentrated H_2SO_4 and one glass bead to each of a series of ten micro-Kjeldahl flasks containing 2, 6, 8, 10, 11, 12, 13, 14, 15 and 16 ml. of the standard NH_4Cl solution (1 ml. $\Leftrightarrow$ 0.01 mg. N), and proceed as in the determination of the reagent blank with the exception that the percentage transmission is measured at 420 mμ with the $K_2Cr_2O_7$ permanent color blank as a reference in place of distilled water. Plot the percentage transmission values obtained against the number of milligrams of nitrogen per 50 ml. of digested solution as indicated in figure 2.

Analytical procedure. Transfer approximately 1 g. of milk, weighed accurately in a weight pipette on an analytical balance, to a 500 ml. volumetric flask and dilute to the mark with NH_3-free water. A 12.5 ml. aliquot of this solution then is transferred to a micro-Kjeldahl flask. The procedure used in establishing the standard curve is followed except that the solution should fade from a dark-brown to a straw-yellow before the timing of the 3 minutes of additional digestion is begun. From the value obtained for the percentage transmission at 420 mμ on the spectrophotometer, the amount of nitrogen present in

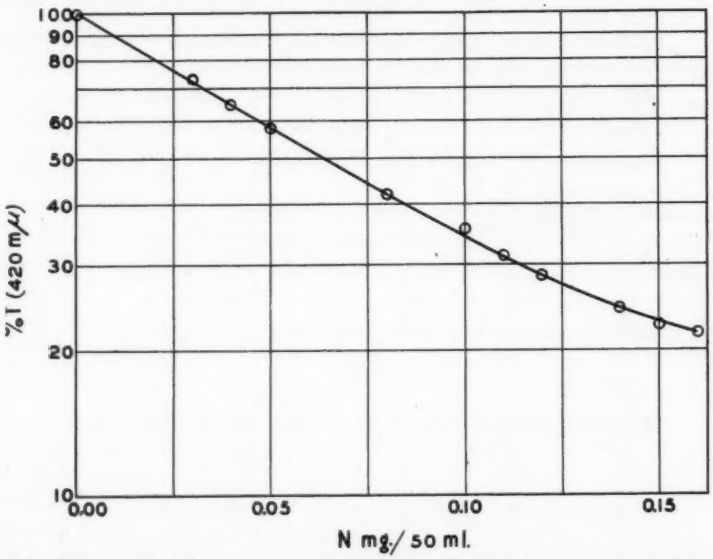

FIG. 2. Relationship between percentage transmission at 420 mμ for the Nessler reagent-ammonia complex at various concentrations.

the aliquot used can be determined from the standard curve. This value in turn can be used to calculate the percentage nitrogen in the sample.

RESULTS

The Relationship Between the Wave Length and the Percentage Transmission of Light by Solutions of the Nessler Reagent-Ammonia Complex. The percentage transmission vs. the wave length curves obtained vary considerably, depending upon the particular Nessler reagent used, the amounts of various reagents present, the concentration of ammonia present, the temperature of color development and the manner of addition of the reagents. This is believed to be due to the colloidal nature of the colored complex formed. These various factors would influence the size and composition of the particles formed. No attempt was made to study these phenomena in detail, but sufficient preliminary work was done to establish the dependence of the color obtained upon the above factors. For the

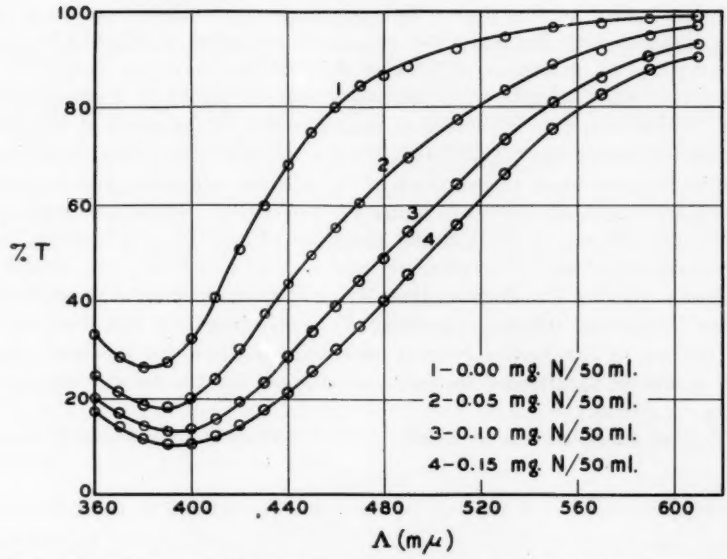

FIG. 3. Percentage transmission vs. wave length curves for the Nessler reagent-ammonia complex at various concentrations.

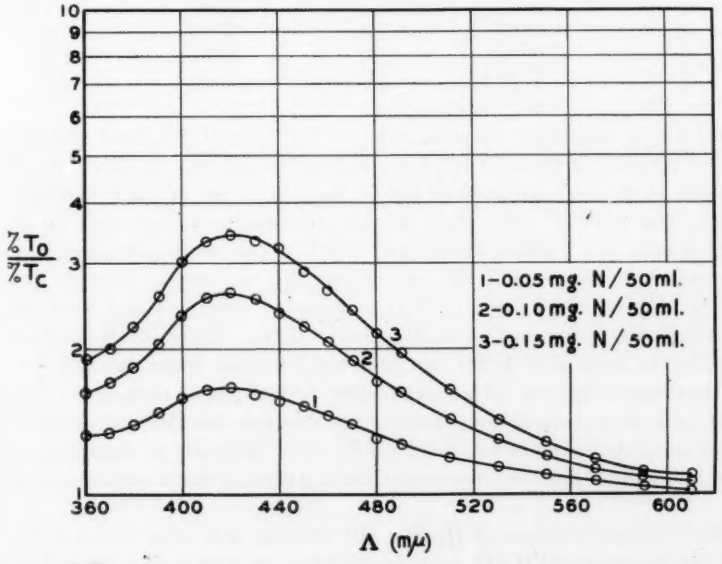

FIG. 4. $\frac{\%T_o}{\%T_c}$ vs. wave length curves for the Nessler reagent-ammonia complex at various concentrations.

particular method in this paper, the percentage transmission vs. wave length curves obtained with distilled water as a blank are given in figure 3 for several concentrations of ammonia. A decided shift in the minimum transmission toward longer wave lengths with increasing concentrations of ammonia can be noted. When this data is plotted as the logarithm of the quotient obtained by dividing the percentage transmission for the solution containing no added ammonia by the percentage transmission of the solution containing added ammonia, vs. the wave length, the curves in figure 4 are obtained. These curves all show a maximum at 420 mμ which indicates the point of maximum absorption for the Nessler reagent-ammonia complex. It also may be noted that the colored complex does not follow the Bouguer-Beer-Lambert Law, since the absorption is not a linear function of the concentration. The wave length of this maximum will be a function of the Nessler reagent used and therefore, for the most accurate work, should be determined by each investigator for the Nessler reagent that he uses.

Effect of concentration of K_2SO_4. Since Self (11) observed that ammonia

TABLE 1

Effect of concentration of K_2SO_4 upon the nitrogen values obtained by direct Nesslerization (0.2244 g. sample of milk)

Sample no.	Wt. K_2SO_4	Transmission (420 mμ)	Concentration	Nitrogen
	(*g.*)	(%)	(*mg. N/50 ml.*)	(%)
1	0.0000	32.9	0.1070	0.463
2	0.1989	32.9	0.1070	0.463
3	0.4024	31.6	0.1115	0.482
4	0.6024	31.8	0.1110	0.480
5	1.0000	40.0	0.0870	0.376

was lost during digestion when the ratio of K_2SO_4 to H_2SO_4 used was favorable for the formation of the acid sulphate, it was thought wise to check the effect of variations in the concentration of K_2SO_4 used upon the amount of nitrogen obtained by this method. Therefore, five separate analyses were made on the same sample of milk with varying amounts of K_2SO_4. The results are given in table 1.

Sample 1, to which no K_2SO_4 was added, did not become straw-yellow until after the addition of 30 per cent H_2O_2. Sample 5 solidified in a hard cake and sputtered badly upon addition of 30 per cent H_2O_2. These results indicate that, as long as the amount of K_2SO_4 added is not less than approximately 0.4 g. nor more than approximately 0.6 g., satisfactory results can be obtained. Therefore, the use of a measuring spoon is sufficiently accurate for the addition of K_2SO_4. Smaller amounts should be avoided because of the difficulty in obtaining complete digestion. Larger amounts may cause loss of ammonia due to acid-sulphate formation.

Effect of concentration of H_2SO_4. In order to determine the accuracy with which the concentrated H_2SO_4 must be measured so that it does not influence the results of the test, four complete analyses were made of the same sample of milk to which varying amounts of concentrated H_2SO_4 had been added. The results are given in table 2.

TABLE 2

Effect of concentration of H_2SO_4 upon the nitrogen values obtained by direct Nesslerization (0.9244 g. sample of milk)

Sample no.	Vol. H_2SO_4 (conc.)	Transmission (420 mμ)	Concentration	Nitrogen
	(ml.)	*(%)*	*(mg. N/50 ml.)*	*(%)*
1	0.1	89.0	0.0112	0.048
2	0.3	32.1	0.1095	0.474
3	0.5	31.8	0.1110	0.480
4	0.7	31.9	0.1105	0.478

From these figures, it can be seen that from 0.3 to 0.7 ml. of concentrated H_2SO_4 is sufficient for complete digestion of the sample. Sample 1 behaved similarly to sample 5 in the above series.

Effect of quantity of 30 per cent H_2O_2. To determine the limits within which the amount of H_2O_2 added must be controlled, four complete analyses were made of the same sample of milk to which varying amounts of 30 per cent H_2O_2 had been added. Table 3 shows the results of this study.

TABLE 3

Effect of quantity of 30% H_2O_2 used upon the nitrogen values obtained by direct Nesslerization (0.9244 g. sample of milk)

Sample no.	30% H_2O_2	Transmission (420 mμ)	Concentration	Nitrogen
	(No. drops)	*(%)*	*(mg. N/50 ml.)*	*(%)*
1	0	32.0	0.1090	0.472
2	1	32.4	0.1085	0.470
3	3	32.0	0.1090	0.472
4	5	32.0	0.1090	0.472

Apparently the amount of 30 per cent H_2O_2 is not an important factor in the determination. However, since in all Kjeldahl digestion procedures encountered in the literature additional catalysts or oxidizing agents are used, it was thought desirable to retain the use of H_2O_2 as an additional precaution against incomplete digestion, even though in this sample it was not necessary.

Effect of concentration of gum acacia. When no protective colloid, such as gum acacia, is used, the ionic strength of the solution to which the Nessler reagent is added is so high that turbidity develops in the sample. In order to

TABLE 4

Effect of concentration of gum acacia upon the nitrogen values obtained by direct Nesslerization (0.9244 g. sample of milk)

Sample no.	Vol. 2% gum acacia	Transmission (420 mμ)	Concentration	Nitrogen
	(ml.)	*(%)*	*(mg. N/50 ml.)*	*(%)*
1	0.0	31.0 (turbid)	0.1120	0.485
2	0.5	32.3	0.1085	0.470
3	1.0	32.0	0.1090	0.472
4	1.5	31.4	0.1110	0.480

check the effect of the concentration of gum acacia upon the nitrogen values obtained by the direct nesslerization method, four analyses of a sample of milk were made with additions of varying amounts of 2 per cent gum acacia solution. The results are shown in table 4.

From these values, it can be seen that 0.5 ml. or more of 2 per cent gum acacia solution is necessary in order to prevent turbidity. There is some indication, although it is within the limits of experimental error, that the gum acacia contains some free ammonia. However, slight errors in measurement of the volume of 2 per cent gum acacia will not affect the results seriously.

Effect of Nessler reagent. Several different formulae for the preparation of Nessler reagent appear in the literature (8, 6). However, only two were tested in this work. The procedure described by Kieferle and Gloetzl (6) was used first, but, after preliminary investigation, the reagent described in "The Standard Methods for Examination of Water and Sewage" (1) was selected due to its ease of preparation and sensitivity. This selection is not to be interpreted to mean that the reagent described by Kieferle and Gloetzl is not satisfactory, but merely indicates the authors' preference. The preliminary work also indicated that wide variations in the character of the color developed could be expected with variations in the concentration of Nessler reagent relative to that of the other ingredients.

Effect of temperature on color development. In preliminary work, difficulty was encountered in reproducing precentage transmission vs. concentration curves on successive days, especially when the room temperature had changed noticeably. Therefore, the effect of temperature upon the color developed was investigated with solutions of four different concentrations of NH_4Cl at three different temperatures each. Figure 5 shows the results obtained. The values for the 0.00 mg. N sample were determined with distilled water as a blank, while, for the other concentrations, the $K_2Cr_2O_7$ permanent color blank was used. Since the accuracy of temperature control was only $\pm 1°$ C., no conclusions should be drawn concerning the form of the function expressing the relationship between the intensity of the color and the temperature of color development. Straight lines have been drawn connecting the values obtained as a first approximation in order to estimate the tolerance permissible in temperature control. Since the accuracy of reading the percentage transmission is ± 0.5 per cent, the temperature should be controlled to at least $\pm 1°$ C.

Effect of time of color development. Preliminary work indicated that the intensity of the color increases with the time of color development. In order to determine the most desirable time and the accuracy to which it should be controlled, several solutions of different concentrations of NH_4Cl were made up in the manner employed in the establishment of the standard curve and the percentage transmission measured at time intervals. Figure 6 gives the results of this study. The color appeared to increase in intensity throughout the 24-minute period during which it was followed. Therefore, 10 minutes was selected as a convenient time interval for color development. From the curves, it can

be seen that it is necessary to control this time to ± 3 minutes for the lowest concentration and to ± 6 minutes for the highest.

Reproducibility of Results and a Comparison with Values Obtained by a Modified Kjeldahl-Gunning-Arnold Method. Several analyses of two different milk samples by the method outlined in this paper and by a macro Kjeldahl method were made. The macro method used was the Kjeldahl-Gunning-Arnold

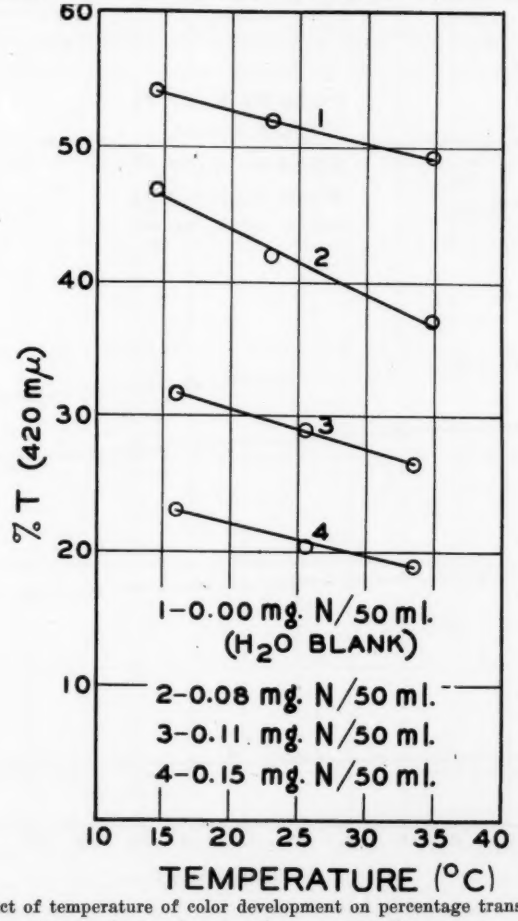

FIG. 5. Effect of temperature of color development on percentage transmission at 420 mμ of the Nessler reagent-ammonia complex at various concentrations.

method outlined in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (2) with the following modifications. Selenized granules were added as an additional catalyst in the digestion procedure, and a boric acid solution containing the mixed indicator, methyl-red and methylene blue, as described by Menefee and Overman (9), was used as a receiver for

the ammonia in place of standard 0.1 N HCl. The ammonia collected then was titrated with 0.1 N HCl to the original gray color of the indicator. These results with their standard deviations are found in table 5.

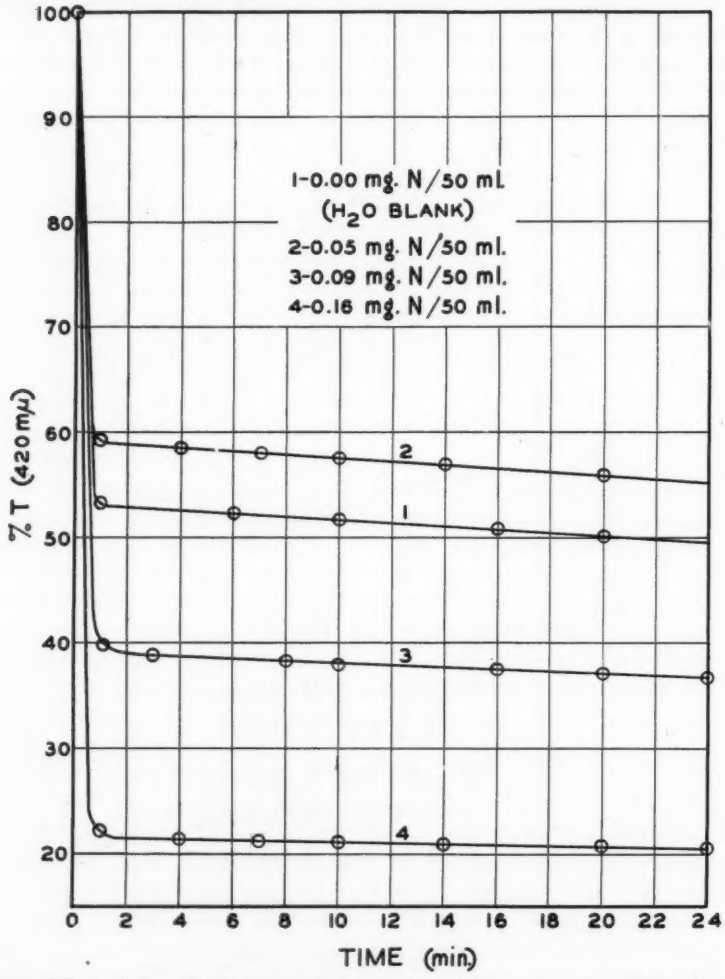

FIG. 6. Effect of time elapsing after Nesslerization on percentage transmission at 420 mμ of the Nessler reagent-ammonia complex at various concentrations.

The percentage deviation for each test from the mean of its series was calculated for the values obtained by direct nesslerization. The optimum standard deviation for these percentage deviations, defined as $\hat{\sigma} = \sqrt{\frac{\Sigma n_i x^2_i}{n-1}}$, was calculated to be 1.52 per cent of the mean nitrogen value. By applying the table for the χ^2

function (13) and the formula, $\chi^2 = \frac{(n-1)\ \hat{\sigma}^2}{\sigma^2}$, it was found that, with 90 per cent certainty, the true standard deviation, σ^2 would not be greater than $\pm$ 1.98 per cent of the mean nitrogen value. From this figure for the true standard deviation and probability tables, it is possible to predict, with 90 percent certainty, that nine times out of ten the results of a single analysis will fall within the limits of $\pm$ 3.25 per cent of the true nitrogen value of the sample. Therefore, the test may be considered sufficiently reproducible for all but the most exacting work.

When compared to the results secured by the modified Kjeldahl-Gunning-

TABLE 5

Comparison of direct Nesslerization method with modified Kjeldahl-Gunning-Arnold method for the determination of nitrogen in the same milk sample

Sample no.	Analysis no.	Direct Nesslerization		Macro Kjeldahl	
		Nitrogen	Deviation	Nitrogen	Deviation
		(%)		(%)	
1	1	0.491	−0.007	0.512	+0.0005
	2	0.490	−0.008	0.511	−0.0005
	3	0.508	+0.010		
	4	0.510	+0.012		
	5	0.495	−0.003		
	6	0.508	+0.010		
	7	0.495	−0.003		
	8	0.505	+0.007		
	9	0.495	−0.003		
	10	0.480	−0.018		
	Mean	0.498	±0.008	0.512	±0.0005
	Standard deviation	±0.009			
2	1	0.482	+0.007	0.491	+0.005
	2	0.480	+0.005	0.481	−0.004
	3	0.472	−0.003	0.486	−0.000
	4	0.472	−0.003		
	5	0.475	0.000		
	6	0.471	−0.004		
	7	0.475	0.000		
	8	0.475	0.000		
	Mean	0.475	±0.003	0.486	±0.003
	Standard deviation	±0.004			

Arnold method, direct nesslerization yields results which are approximately 2.5 per cent low, on the average. By employing the Student's "t" function, $t = \frac{(\overline{X}_1 - \overline{X}_2)}{\sqrt{n_1 s^2_1 + n_2 s^2_2}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$, and consulting appropriate tables (12), this observed difference is found to be significant. However, this deviation is within the experimental error of a single determination and, therefore, would not constitute a serious objection to the method. No explanation for this deviation has been established as yet, but Erdmann (4) has observed that some of the nitrogen present in certain organic materials is converted to the amines instead of to

ammonia. These amines would be titrated as ammonia in the Kjeldahl-Gunning-Arnold method, but would not develop the same color with the Nessler reagent. This may account for the discrepancy between the results of the two methods.

SUMMARY

A rapid method for the determination of nitrogen in milk by direct nesslerization of digested sample has been described.

The effect of the variables involved in the test has been investigated and their tolerance established.

When compared with a modified Kjeldahl-Gunning-Arnold method for the macro determination of nitrogen, the method yields results which are approximately 2.5 per cent low on the average, but are within the experimental error.

The method yields reproducible results which are sufficiently accurate for all but the most exacting work, with a considerable saving in time and reagents when compared with other methods currently in use.

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SOME PHYSICAL AND CHEMICAL PROPERTIES OF BOVINE SALIVA WHICH MAY AFFECT RUMEN DIGESTION AND SYNTHESIS[1]

J. T. REID[2] AND C. F. HUFFMAN

Institute of Nutrition and Dairy Department, Michigan State College

The early studies of Pavlov (25) placed great significance on the role of saliva mainly as that of a watery secretion. Although it generally is accepted that the high water content of saliva is a digestive aid as a lubricant, solvent and medium, other constituents and properties of saliva appear to be of equal importance in rumen digestion. Doubtless some of these substances and properties exert an influence on the activity of rumen flora and fauna.

Scheunert and Trautmann (30) found that the water content of parotid and mandibular saliva of sheep ranged from 98.75 to 98.90 per cent and 99.0 to 99.6 per cent, respectively. The submaxillary saliva of the cow (19) and the parotid saliva of the buffalo (33) were found to contain 99.11 and 99.15 per cent water, respectively.

The following figures were reported for the pH of saliva of several ruminant species: buffalo, 8.8 (33); sheep, 8.12 to 8.32 (28); goats, 8.2 to 8.8 (2); calves, 8.1 (32, 38) and 8.23 (31); and cattle, 8.55 to 8.90 with an average of 8.8 (10). Chrzaszcz and Schechtlówna (10) observed a decrease in the pH of saliva to 7.9 during rumination.

Some reducing properties and several associated constituents of saliva have been investigated by several workers. Suzuki (36) reported that human saliva generally contains less than 0.18 mg. per cent ascorbic acid, and that age and sex are without effect upon the concentration of this substance. This investigator was not able to detect ascorbic acid in oxidized form in human saliva or in the salivary glands of rabbits. The ascorbic acid content of the salivary glands was found to be progressively lower in the species of the following orders: rodentia, carnivora and ungulata (36). Suzuki (36) demonstrated an increase in the ascorbic acid content of the parotid glands of the rabbit by injecting 2 mg. of ascorbic acid per kg. of body weight or by administering pilocarpine hydrochloride; the latter substance, however, did not alter the concentration in the submaxillary glands. The investigations of Zimmet and Dubois-Ferriere (40) revealed that the concentration of ascorbic acid in children's saliva increased progressively with age. Levels of 0.04 mg. per cent for 4-year-old children, 0.11 mg. per cent for 16-year-olds, and a marked lowering of the saliva ascorbic acid level in children under febrile conditions were reported (40). These results, relative to the age effects, are contrary to those of Suzuki (36). Held *et al.* (14) believed 0.12 to 0.16 mg. per cent to be the normal ascorbic acid

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[2] Present address: Department of Animal Husbandry, Cornell University, Ithaca, New York.

content of human saliva. Concentrations of 0.04 to 0.08 mg. per cent were found in the saliva of patients suffering from paradentoses (14). A higher normal level (0.25 mg. per cent) was reported by Stuteville (35). Suzuki (37) showed that the glutathione content of the parotid and submaxillary glands of scorbutic guinea pigs was 12.2 to 26.2 per cent and 16.5 to 17.4 per cent lower, respectively, than those of normal guinea pigs. Pilocarpine decreased the glutathione content of these glands in rabbits, whereas atropine was without effect. Injections of iodoacetic acid or sodium iodoacetate resulted in the complete disappearance of reduced glutathione from these glands. Pincus (26, 27) demonstrated the reduction of several dyes by saliva.

Very little attention has been given to the physical or physico-chemical properties of saliva. However, in his study of surface energy of some physiological fluids, Wwedensky (39) found that the lowering of surface tension by saliva was considerable and classified it with bile and milk, as a surface active fluid. In a later study of surface activity of human saliva, Borissovsky and Wwedensky (6) found that the secretion stimulated by acids manifested a surface tension-temperature curve characteristic of surface inactive fluids, whereas a curve characteristic of surface active substances was demonstrable for saliva stimulated by food material. These studies suggest that saliva evoked by acid solutions is a protective reaction, while that produced as a result of food excitation is a normal secretion. Simultaneous studies conducted on the relative adsorption by the salivas produced by these two methods of stimulation showed typical adsorption by the food-stimulated saliva, whereas adsorption curves characteristic of surface inactive substances were obtained for the acid-stimulated secretion.

Fomin (13) reported that the saliva of calves varies considerably in viscosity and that the phenomenon appears attributable to the consumption of different types of feed. Hoepfner (15) demonstrated a pronounced structure-viscosity in human saliva which persisted for 24 hours despite definite chemical changes. Curves which are characteristic of structure-viscous liquids were obtained in this investigation by plotting the differential flow-velocity against time. Fresh saliva was converted completely into foam and had a foaming capacity equivalent to that of a 0.02 per cent saponin solution.

Recognition of literature has been limited here to reports dealing primarily with the saliva of ruminant animals and to studies of saliva in other animals and human beings which are pertinent to certain phases of the study summarized in this report.

The object of this investigation was to probe some of the chemical and physico-chemical properties of bovine saliva which may influence rumen digestion and synthesis.

EXPERIMENTAL PROCEDURE

During the course of this investigation, study was made of 77 samples of saliva collected from the unstimulated secretion (except for tactile, visual and auditory stimulation) of 42 Guernsey, Jersey, Holstein and Brown Swiss cattle

of varying ages. Fifty-four samples were collected under neutral paraffin oil to prevent carbon dioxide loss, while 23 samples were taken without oil protection. Blood samples were drawn at the time of saliva collection for ascorbic acid analysis.

The pH of 77 saliva specimens was determined using a Beckman pH meter equipped with a glass electrode. Ascorbic acid was measured in 75 samples according to the method outlined by Mindlin and Butler (24) with modifications (29). The water content of 36 specimens was determined by drying to constant weight. Ascorbic acid analyses were made on corresponding blood plasma samples. The surface tension of 55 samples of saliva was measured by a torsion-bal-

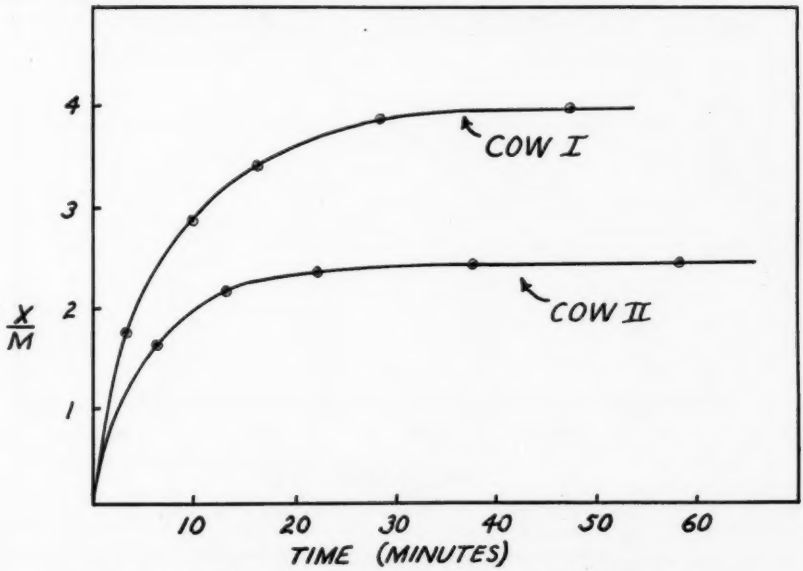

FIG. 1. Adsorption of benzene vapor on the saliva of 2 different cows.

$\frac{X}{M}$ = mg. benzene vapor adsorbed per g. saliva.

ance method in which a Du Nouy tensiometer was employed. The deodorizing capacity of saliva was tested by comparing the odor evolved from various quantities of finely powdered garlic suspended in saliva to that evolved by garlic in distilled water. The possibility of saliva possessing an adsorptive property was investigated by passing benzene at constant temperature (27° C) and pressure (3 bubbles per second) through a U-type adsorption tube containing known quantities of cow's saliva. The amount of benzene taken up by the saliva after exposure for different time intervals was determined by weight. The $\frac{X}{M}$ values (amount of benzene taken up per unit weight of saliva) were plotted against the time exposed (fig. 1).

Similar experiments were conducted in which dry, powdered saliva taken from 26 cows (fig. 2) was employed in place of fresh natural saliva, and in which distilled water was substituted for saliva.

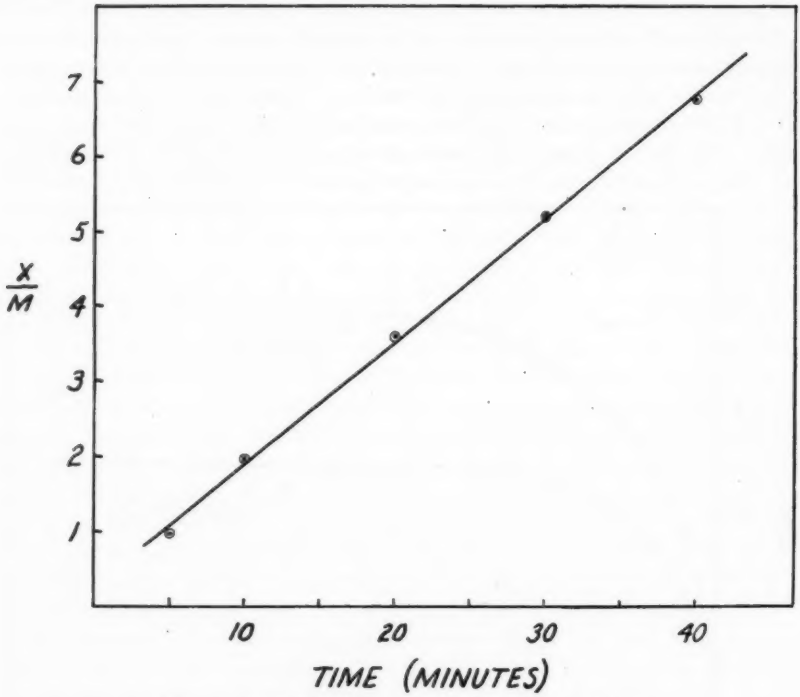

FIG. 2. Absorption of benzene by dried saliva of cows.

$\frac{X}{M}$ = mg. benzene vapor absorbed per g. dried saliva.

RESULTS

The average pH of 23 saliva samples not collected under oil was 8.71, whereas that for 54 samples protected by oil from carbon dioxide loss was 8.53. Some representative values for each are shown in tables 1 and 2. These data suggest that it is imperative to record the conditions under which saliva is collected from animals, *i.e.* whether or not the specimens are protected from carbon dioxide loss. No great variation was observed in the pH of saliva collected over a 24-hour period, and the time of collection relative to the feeding time had no noticeable effect on the reaction of the saliva.

An analysis of 75 saliva samples revealed an average concentration of 0.15 mg. per cent ascorbic acid, while plasma samples from the corresponding animals at the time of saliva collection had an average ascorbic acid content of 0.47 mg. per cent. Table 2 shows that the ascorbic acid content of saliva collected at intervals

of 6 hours over a 24-hour period was variable and bore no appreciable relationship to the plasma ascorbic acid level. The correlation coefficient between the ascorbic acid level of the plasma and saliva was + 0.015.

The average surface tension of 55 saliva samples was 47.1 dynes per cm. at 29° C. Data obtained from the benzene uptake by fresh saliva which had been exposed for varying time periods derived typical adsorption curves (fig. 1), which indicate that saliva contains adsorptive substances. However, when a similar experiment was conducted on dry, powdered saliva taken from 26 cows, a curve was obtained from the benzene up take which is suggestive of absorption (fig. 2). It seemed, therefore, that the adsorptive property of natural saliva is destroyed by drying.

When water was substituted for saliva in a similar trial, weight losses were noted which were attributed to evaporation.

TABLE 1

Representative data on some chemical and physical properties of cow's saliva and the plasma ascorbic acid level

Animal no.	Plasma	Saliva			
	Ascorbic acid	Ascorbic acid	Water	Surface tension	pH
	(*mg. %*)	(*mg. %*)	(%)	(*dynes/cm.*)	
A5	0.55	0.19	99.10	48.07	8.55
A18	0.42	0.13	98.83	46.05	8.50[a]
A31	0.72	0.07	99.23	47.97	8.80
A46	0.49	0.06	99.60	45.54	8.62[a]
A49	0.62	0.16	98.93	46.96	8.56[a]
B58	0.59	0.13	98.70	47.46	8.63[a]
C553	0.20	0.02	.....	49.20	8.76
127	0.37	0.21	99.01	45.64	8.93

[a] Samples collected under neutral paraffin oil.

It was found that 60 mg. of powdered garlic suspended in 100 ml. of distilled water evolved the pungent garlic odor, whereas no characteristic garlic odor was detected by 20 innocent persons from the same amount of the substance suspended in 100 ml. of cow saliva. The elimination of odors by saliva was considered to be an adsorption phenomenon in view of the great surface activity of bovine saliva.

DISCUSSION

The early studies of the role of saliva in digestion concerned the importance of this secretion as a mechanical digestive aid by virtue of its ability to moisten, dissolve and lubricate the dry feeds which constitute the cow's ration. Recent interest in the chemistry and physiology of rumen fermentation and synthesis gave impetus to a study of the constituents and properties of saliva which may influence numbers and kinds of microorganisms.

Greater importance now is being attached to the buffering ability of ruminant saliva than formerly. The large quantity of saliva secreted, its reaction and its activity as a buffer on the alkaline side of a pH of 4 are believed to be largely responsible for the maintenance of a medium which appears to be optimal for micro-

TABLE 2

Blood plasma and saliva ascorbic acid and saliva pH at intervals during a 24 hr. period

Time	Ascorbic acid (mg. %)										Saliva pH[d]				
	Plasma					Saliva									
	A33	A44	A50	419	C553	A33	A44	A50	419	C553	A33	A44	A50	419	C553
A.M. 12: 30–1: 30	0.59	0.41	0.56	0.68	0.36	0.11	0.18	0.20	0.06	0.03	8.54	8.62	8.48	8.51	8.57
A.M. 6: 40–7: 30[a]	0.53	0.22	0.50	0.42	0.20	0.16	0.03	0.13	0.14	0.02	8.53	8.53	8.56	8.60	8.76
P.M. 12: 00–12: 45[b]	0.58	0.35	0.42	0.39	0.27	0.13	0.14	0.05	0.11	0.05	8.57	8.45	8.60	8.41	8.48
P.M. 6: 10–6: 35[c]	0.55	0.30	0.60	0.69	0.37	0.09	0.03	0.27	0.27	0.03	8.53	8.46	8.40	8.50	8.49

[a] Samples collected before morning feeding.
[b] Samples collected before afternoon feeding.
[c] Samples collected after afternoon feeding.
[d] Samples collected under neutral paraffin oil.

bial activity and for the chemical changes occurring in the rumen. The saliva secreted by a mature animal during 24 hours contains approximately 300 to 350 g. of sodium carbonate according to Colin (11), which is theoretically capable of neutralizing 56.6 to 65.0 liters of 0.1 N hydrochloric acid solution.

The alkalinity of dairy cattle saliva offers the possibility that it might absorb some of the carbon dioxide produced in the rumen (22). The increased amount of saliva secreted at the time of eating, regurgitation and rumination may be nature's compensation for the great amount of fermentation during and immediately following eating.

A cow secreting 56 liters of saliva daily excretes approximately 86 mg. of ascorbic acid by this route. Although no explanation has been proposed for the significance of this saliva constituent, the possible use of the substance in saliva could be theorized purely on the basis of its action in other biological systems: (a) the control of hydrolytic enzyme systems, (b) the regulation of oxidation-reduction systems, since it is known to function in H transfers, (c) the detoxification of chemicals such as lead and arsenic by combining with these substances, (d) the initiation of the synthesis of some proteins, since it is known that certain amino acids such as leucine are dehydrated to form ammonia and keto-acids, (e) the inhibition of some organisms which may otherwise be pathogenic to the mouth, esophagus and lower digestive tract, and (f) the encouragement or stimulation of fermentation by certain organisms, since it is known that some of the microorganisms inhabiting the rumen use vitamin C in their metabolism.

To the saliva of the cow has been credited the deodorizing of undesirable barn odors, and hence the elimination of some of the off-flavors of milk without a known explanation for the deodorizing process. A comparison of the odor of garlic suspended in water and saliva demonstrated that no characteristic odor was evolved from the saliva while a pungent odor was readily detected from garlic in water. Since the deodorizing phenomenon generally is regarded as one of adsorption, as shown in water-odor purification studies by Baylis (4, 5) and Spalding (34), a phase of this investigation was given to the study of the surface activity of saliva. Natural saliva demonstrated typical adsorption tendencies, whereas dried saliva gave results characteristic of absorption. It is known that a considerable portion of the solid matter in saliva is protein; Bramkamp (8) found 250 mg. per cent protein, and Inouye *et al.* (16) reported that saliva contains 260 mg. per cent mucinate. Zolnikova (41) reported that 86 to 89 per cent of the total nitrogen of saliva is mucin nitrogen. According to Bucher (9), the mucoid proteins of gastric mucus are extremely small particles which are not visible using the ultramicroscope, but are amorphous-like in nature and possess colloidal properties. Since Levene (20) has shown that the protein complex from one mucin to another differs chiefly in the nature of their amino-hexoses, it appears that the mucoid proteins of saliva also are of small molecular size which would facilitate adsorption by virtue of their great surface area. Mahlo (21) stated that the hydrochloric acid of gastric juice is adsorbed partly by mucin. Investigations by Bradley (7) and Babkin (3) demonstrated the capability of

mucin as an adsorbent. From an analysis of the foregoing studies it seemed logical to attribute the adsorption properties of cow's saliva to mucoid proteins.

Although the uptake of benzene by saliva was in small quantities, the same test applied to water showed a loss of weight even when exposed only one minute and progressive decreases thereafter which seemed to be attributable to evaporation. The amount of benzene taken up by the saliva could not be accounted for by solubility, because the quantity of benzene which can be dissolved in the amount of water contained in the saliva (0.70 mg. of benzene per g. of saliva at 22° C.) is a very small portion of the amount actually adsorbed during any time interval.

The low surface tension (47.10 dynes per cm.) of dairy cattle saliva is an amazing fact when one perpends its high water content (99.12 per cent) or compares it to a well known surface tension depressant such as a 0.1 per cent sodium oleate solution which has a surface tension of 42.2 dynes per cm. and to pure water with a surface tension of 71.35 dynes per cm. at 30° C. This property, indicating wetting ability, offers a plausible account for the efficiency of saliva in moistening feed materials.

McCulloch (23) stated that the surface tension of bacterial cultural media usually is 50 to 60 dynes. Larson *et al.* (17, 18) showed that the amount of growth of certain bacteria depends to a great extent upon the surface tension of the media. Albus and Holm (1) reported that *Lactobacillus acidophilus* grew well at a surface tension of 36 dynes, whereas *Lactobacillus bulgaricus* could not initiate growth in a medium whose surface tension was reduced to 40 dynes per cm. These workers (1) believed that a low surface tension is responsible for the failure to obtain a permanent implantation of *L. bulgaricus* in the normal human intestinal tract, whereas *L. acidophilus* implantations were successful. Probably the low surface tension of saliva is optimal for the bacteria types indigenous to the rumen and may be selective to the extent of limiting the kind of organism present.

In view of the surface energy exerted by the large quantity of saliva secreted by the cow, the efficiency of chemical reactions and bacterial functions occurring in the rumen by virtue of wetting, solution and mixing, in addition to maintaining the proper rumen reaction, seem responsible to saliva activity.

SUMMARY

An investigation was conducted on 77 samples of saliva from dairy cattle to ascertain some of this secretion's chemical and physico-chemical properties. The average water content of 36 specimens was 99.12 per cent.

The average pH of 54 samples protected by neutral paraffin oil from carbon dioxide loss was 8.53, whereas the average pH for the unprotected samples was 8.71. No appreciable variation was noted in the pH of saliva collected at 6-hour intervals over a 24-hour period, and the time of collection relative to the feeding time had no apparent effect on the reaction of the saliva.

An analysis of 75 saliva samples showed an average ascorbic acid concentra-

tion of 0.15 mg. per cent, while the average plasma ascorbic acid level of the same animals was 0.47 mg. per cent.

The deodorizing and surface active properties of cow's saliva were demonstrated. The surface tension of 55 samples was 47.10 dynes per cm. at 29° C., while typical adsorption curves were derived from the uptake of benzene by saliva. Since dried cow's saliva took up benzene in a manner characteristic of adsorption, it was believed that the adsorbent materials of saliva were altered in some way by drying, resulting in the loss of the adsorbing property.

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THE INFLUENCE OF TOCOPHEROLS AND COD-LIVER OIL ON MILK AND FAT PRODUCTION

FRANK WHITING, J. K. LOOSLI, VLADIMIR N. KRUKOVSKY, AND K. L. TURK

Departments of Animal Husbandry and Dairy Industry, Cornell University, Ithaca, N. Y.

Numerous studies have shown that feeding cod-liver oil to dairy cows and goats markedly reduces the fat percentage of the milk (7, 8, 21, 24). Little is known about the specific cause of this depression of the fat test, but it has been shown that hydrogenated cod-liver oil is no longer active (8, 22). Moore *et al.* (23) observed that giving cod-liver oil to cows in 12 feedings each day caused no such marked depression of test in a 6-day feeding period as when the oil was fed in a single daily dose. Certain other fats and oils have been shown to cause an increase, at least temporary, in the fat content of the milk of cows and goats (16).

Recently, Harris *et al.* (10) have reported that the percentage of fat in the milk of dairy cows was increased approximately 27 per cent by feeding 1 g. of mixed natural tocopherols daily over a 20-month period.

The foregoing reports and the established antagonistic action between cod-liver oil and alpha-tocopherol in nutritional muscle dystrophy (19) led the authors to wonder if a similar interrelationship might exist in the secretion of milk fat by cows. Accordingly, an experiment with cows was designed to study further the effect of tocopherols upon milk production and particularly to determine whether tocopherols would inhibit the depressing influence of cod-liver oil on the fat percentage.

EXPERIMENTAL PROCEDURE

Sixteen cows of the Holstein, Brown Swiss and Guernsey breeds were used in this study during the winter of 1947–48. The cows were divided into four equal groups according to age and breed. The first group consisted of mature Brown Swiss cows; the second group, 2-year old Brown Swiss cows; the third group, 2-year old Holstein cows and the fourth group, mature Guernsey cows. Four replicates of 4×4 Latin squares were used. Control ration A consisted of medium quality mixed grass and legume hay, corn silage and a commercial 16 per cent protein dairy concentrate mixture. Ration B was the control ration plus 1 g. of mixed natural tocopherol.[1] Ration C was ration A plus 5 oz. veterinary grade cod-liver oil.[2] Ration D consisted of ration A plus 5 oz. veterinary grade cod-liver oil and 1 g. of mixed tocopherols.

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[1] A stabilized tocopherol concentrate "Myvadry" purchased from Distillation Products, Inc., Rochester, N. Y.

[2] Contained 2000 units of vitamin A and 100 units of vitamin D per g. Purchased from Marine Products Co., Boston, Mass.

One lb. of grain was fed for each 3 lb. of 4 per cent fat-corrected milk produced during the preliminary period. During the experiment, the grain allowance was reduced in accordance with the average decline in production of all cows as recommended by Lucas (17). A constant amount of silage, approximately 25 lb. daily per cow, was fed throughout the experiment. Hay was fed ad libitum.

The tocopherol supplement was placed on top of each cow's morning feeding of concentrate. Cod-liver oil was mixed with a small amount of molasses and poured on the silage at the evening feeding. The cod-liver oil and tocopherols were fed at separate feedings, because Mackenzie *et al.* (19) had shown that the muscular-dystrophic preventative action of tocopherol was rendered ineffective when it was administered with cod-liver oil.

Each cow was started on experiment approximately 60 days after parturition, following a preliminary period of 3 weeks, thus all cows were in the same stage of lactation. Each period was of 4 weeks duration and was followed by 1 week on the control ration before starting the next period.

Milk was weighed and sampled at each milking. These aliquots were composited for seven milkings, and butterfat determinations by the Babcock test were made on the composited aliquots twice weekly. At the end of each 4-week period, samples of blood were obtained for tocopherol, vitamin A and carotenoid analyses. A 2 quart sample of milk was taken from the morning milking at the end of each 4-week period for studies on tocopherol, vitamin A and carotene content and keeping qualities.

The method of Koehn (14) was used to measure the vitamin A and carotenoid content of milk fat, while that of Kimble (13) was used to determine the vitamin A and carotenoid content of blood plasma. The procedure of Quaife (25) was used to determine the tocopherol content of milk fat and that of Quaife and Harris (27), with the microhydrogenation apparatus of Quaife and Biehler (26), to determine the tocopherol content of the blood plasma.

In an additional study, six Holstein cows were used in a single-reversal experiment during the summer of 1947 to study the influence on milk and fat production of feeding 1 g. daily of mixed natural tocopherols to cows on pasture. In this experiment, the cows were divided into two groups of three cows each; the two groups were equalized in daily milk production, state of lactation and fat test. The supplemental periods were of 5 weeks duration. The cows were on good pasture and received a concentrate allowance in accordance with milk production (1 lb. of concentrate per 4 lb. of milk produced). The method of feeding the supplement and of handling the milk was essentially the same as described above.

RESULTS

The average daily pounds of milk produced, butterfat tests, pounds of fat produced and the pounds of 4 per cent fat-corrected milk (6) are presented in table 1. Only the milk and fat yields obtained during the last 2 weeks of each period were used in calculating these averages, since the maximum fat depress-

ing effect of cod-liver oil was not evident until the second week; also Harris *et al.* (10) reported that the stimulating effect of tocopherol on fat percentage was not evident until approximately 10 days after starting to feed the supplement. In the experiment with the cows on pasture, milk and butterfat yields during the last 3 weeks were used to calculate averages, since the periods in this experiment were of 5 weeks duration.

Feeding 1 g. of mixed tocopherols daily over a 4-week feeding period slightly increased the fat test, the average daily lb. of milk and fat produced, and the average daily pounds of 4 per cent fat-corrected milk (table 1). However, none of these increases was statistically significant. These results do not confirm the findings of Harris *et al.* (10), but it should be emphasized that the length of the feeding period in this experiment was only 4 weeks (5 weeks on pasture), whereas Harris *et al.* used a 20-month experimental period.

TABLE 1

The influence of tocopherols and cod-liver oil upon the average butterfat percentage, total milk yield and 4% fat corrected milk

Supplement	Butterfat	Average daily production		
		Milk	Fat	4% F.C.M.
	(%)	(*lb.*)	(*lb.*)	(*lb.*)
	Winter, 1947–48			
None	4.24	30.3	1.28	31.4
Tocopherols	4.30	30.7	1.32	32.1
Cod liver oil	3.63	32.8	1.19	31.0
Tocopherols and cod-liver oil	3.63	32.0	1.16	30.3
	Summer, 1947[a] (pasture)			
None	3.55	35.7	1.27	33.3
Tocopherols	3.58	35.2	1.26	33.0

[a] Holstein cows only.

Our results are in agreement with those of Gullickson *et al.* (9), who observed no increase in the fat yield or fat percentage from feeding tocopherols.

Feeding 5 oz. of cod-liver oil alone or with tocopherol caused a 14 per cent decrease in the average fat test, a slight decrease in average daily pounds of 4 per cent fat-corrected milk, but a 6 per cent increase in average daily pounds of milk produced. The decrease in fat test and the increase in milk yield were both highly significant (1 per cent level of probability), using the method of Cochran *et al.* (3) to analyze the data. The fat depressing effect of cod-liver oil observed in this experiment agrees with the findings of others, but no one has reported an increase in milk yield due to feeding cod-liver oil. Petersen (24) and McCay and Maynard (21) reported that milk production was not affected by cod-liver oil feeding. It has been shown previously that feeding extra vitamin A does not increase milk production (12, 18). No explanation is offered for the increase in milk production found in these investigations from feeding cod-liver oil.

Feeding tocopherols did not prevent the drop in fat test due to feeding cod-liver oil. Apparently the supplemental tocopherol was not destroyed by the cod-liver oil, since the tocopherol content of the butterfat and the blood of the cows when receiving tocopherol and cod-liver oil were essentially the same as when they received tocopherol alone (table 2). Feeding cod-liver oil alone, however, did lower the tocopherol content of the butterfat and milk, indicating that cod-liver oil destroys tocopherols in the dairy cow in a manner similar to that observed in other animals.

Cod-liver oil depressed the carotene content of the blood and milk fat and increased the vitamin A content. A similar phenomenon has been observed by other investigators when high levels of vitamin A have been fed (2, 5, 10, 29). However, when both tocopherols and cod-liver oil were fed, the carotene-depressing effect of the cod-liver oil was not so pronounced. Harris *et al.* (10) noted that feeding tocopherols with a vitamin A concentrate largely prevented the carotene depressing effect of the vitamin A supplement.

TABLE 2

The tocopherol, vitamin A and carotenoid content of milk fat and blood plasma as affected by tocopherol and cod-liver oil supplements

Supplement	Content in milk fat of:			Content in blood plasma of:		
	Tocopherols	Vitamin A	Carotenoids	Tocopherols	Vitamin A	Carotenoids
		(γ/*100 g.*)			(γ/*100 ml.*)	
None	2990	559	440	582	27.8	528
Tocopherols	3569	544	390	735	28.8	463
Cod-liver oil	2529	1301	326	427	31.5	368
Tocopherols and cod-liver oil	3590	1329	349	696	34.3	438
None[a]	3190	.....	.....	685	22.2	1054
Tocopherols[a]	3640	.....	.....	771	23.9	1106

[a] Holstein cows only.

Of special interest is the fact that tocopherol feeding improved the ability of milk to resist the development of off-flavors as described in detail by Krukovsky *et al.* (15).

SUMMARY

Feeding 1 g. of natural mixed tocopherols daily to dairy cows during both winter and pasture feeding for periods of 4 to 5 weeks had no significant influence on milk or fat production. However, feeding 5 oz. of cod-liver oil daily to cows during winter feeding decreased the butterfat percentage approximately 14 per cent, but increased the total milk production 6 per cent. Tocopherol supplementation did not counteract the butterfat depressing effect of cod-liver oil.

The tocopherol content of the milk fat and blood plasma was increased by feeding tocopherols, but was decreased by feeding cod-liver oil. Cod-liver oil had a depressing effect on the carotene content of the milk fat and blood plasma,

but this was counteracted somewhat by feeding tocopherols with the cod-liver oil. The vitamin A content of the milk fat and blood plasma was increased by feeding cod-liver oil.

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EFFECTS OF LACTOSE ON GASTRO-INTESTINAL MOTILITY: A REVIEW[1]

JESSIE E. FISCHER AND T. S. SUTTON
Institute of Nutrition and Food Technology, and The Department of Dairy Husbandry, The Ohio State University, Columbus

INTRODUCTION

The solids of milk are approximately 38.5 per cent carbohydrate, and the nonfat milk solids are approximately 51 per cent carbohydrate. This carbohydrate (lactose) is unique in that it yields glucose and galactose in equimolecular quantities on hydrolysis. The literature dealing with the commercial preparation, chemical properties and industrial uses of lactose has been reviewed recently (100).

In the study of the nutritive value of milk and dairy products, lactose has received less attention than the protein, fat, vitamin and mineral constituents. In numerous investigations, the lactose of milk is simply considered as so much carbohydrate without cognizance of any special characteristics, so that in some instances nutritive differences attributed to other factors may well have resulted from differences in lactose content of the diets under observation.

Since lactose constitutes a major portion of nonfat dry milk solids and since there is abundant evidence indicating special dietary characteristics of this carbohydrate, further investigation of its nutritive value appears to be justified. One of these special dietary characteristics of lactose is its effect on the movement of food residues through the alimentary tract. As a background for further investigation of this special and perhaps important dietary characteristic of lactose, this literature review is prepared.

THE LAXATIVE NATURE OF LACTOSE

Numerous investigators have reported the occurrence of diarrhea when various amounts of lactose were fed. In most of these reports, no attempts were made to ascertain the approximate lowest level of lactose feeding at which a laxative effect first was observed. The reports of the occurrence of diarrhea generally were made as interesting side lights of investigations which had been designed for different primary purposes, and for this reason the percentages of lactose in the diets had been determined previously to suit these other purposes. Therefore, the following reports of amounts of lactose which produced diarrhea should not be taken to indicate the smallest consumption which is capable of initiating laxation, unless it is so stated.

Kittens were observed by Mitchell and Dodge (62) to suffer such severe diarrhea on a ration containing 70 per cent of lactose that this level of feeding could not be continued.

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Sisson (88) observed that from 10 to 15 per cent of lactose added to a cow's-milk diet produced diarrhea in some puppies but not in others. Younger puppies developed diarrhea sooner than did older ones. The diarrheal condition was alleviated quickly by omitting the lactose from the milk mixture. Gross (36) was not able to feed diets containing more than 15 per cent of lactose without diarrhea appearing, and even the 15 per cent level produced diarrhea in about half of the dogs used. Dragstedt and Peacock (23) observed that the feces of 2 to 3-months-old puppies became liquid in from 4 to 6 days, when a diet of white bread and whole milk was fed with 60 g. of lactose per day. Adult dogs fed a diet of 500 g. of rice, 100 g. of beef heart and 50 g. of lactose did not have liquid feces, but when fed white bread and skim milk *ad libitum*, plus 50 to 125 g. of lactose, most of the adult dogs developed liquid feces after 1 week. This difference in the fluidity of the feces, depending upon the constitution of the diet to which the lactose was added, perhaps may be due to the other constituents of the diet either having some direct effect on fecal consistency or influencing the lactose effect. Diarrhea was noted by French and Cowgill (32) when diets containing 20 per cent of lactose were fed to dogs.

That the rat apparently is better able to utilize lactose than the dog was the conclusion reached by Koehler and Allen (49). They did not observe diarrhea when 35 per cent of lactose was fed to young, full-grown rats. But others have observed diarrhea in adult rats fed diets containing 67 per cent of lactose (85) and 87.5 per cent of lactose (35).

Outhouse *et al.* (67) did not observe diarrhea in their young rats which were fed 25 per cent of lactose in the ration. Whittier *et al.* (101) observed diarrhea in rats at 45 and 63.5 per cent levels of lactose feeding. Mitchell and Dodge (62) reported that diarrhea was exhibited by young rats placed on a 70 per cent lactose ration. Mitchell (60) confirmed this observation for all rat strains used by her, and noted that 35 per cent of *galactose* in the ration did *not* produce the diarrheal effect. Mitchell *et al.* (56) also showed that rations containing 60 to 70 per cent lactose produced diarrhea and slow growth.

Mitchell *et al.* (61) observed diarrhea in young rats placed on a 50 per cent lactose diet. This diarrhea varied in severity with the individual rat. Coryell and Christman (17) stated that, due to the laxative action, lactose doses for rats cannot be increased much beyond 2 g. per kg. of body weight. This figure represents a considerably lower level of lactose intake for producing laxation than has been reported by any other investigator, but it should be noted that Coryell and Christman worked with rats fasted for 24 hours. Diarrhea was observed by Boutwell *et al.* (10) for the first 6 to 12 days of feeding a ration containing 48 per cent lactose and 28 per cent corn oil to weanling rats.

Diarrhea followed by death of rats fed large amounts of lactose in the ration was reported by Evans and Burr (28). Others who have reported a similar occurrence are Fisher (30), Morgan *et al.* (63), and Skeggs and Wright (89), when they fed rations containing 77, 65, and 61.8 per cent of lactose, respectively. Ershoff and Deuel (26) reported that weanling rats fed a ration containing 73.2

per cent lactose or β-lactose developed a severe diarrheal condition and died. The length of time these rats survived depended upon the severity of the diarrhea. The β-lactose rats died sooner than did the lactose rats, having suffered more severe diarrhea in general than had the lactose rats. Attempts to control the diarrhea by including 5 or 10 per cent of citrus pectin or 5 per cent of apple powder in the diet were not successful.

Scott and Verney (86) noted severe diarrhea in rats fed a 62 per cent lactose diet. They found that when rats were allowed to choose dietary components, lactose was avoided, while other carbohydrates were accepted to various degrees. Richter (78) found that rats offered lactose as the sole food ate very small quantities. The rats survived only 7 days on the average, as compared with 4 days for a comparable group given only water. Another group fed only galactose lived only 7 days. When Ershoff (25) fed lactose to rats as the sole food, all the rats had diarrhea, and they died after the same length of time (5 to 7 days) as if they had been fasted. Other rats fed dextrose, sucrose or butter fat as the sole food lived 34 days on the average. That the diarrhea and early deaths were not due to galactose was shown by feeding a ration composed of 50 per cent dextrose and 50 per cent galactose, in which case no diarrhea was observed and the rats lived about as long as the rats fed dextrose, sucrose or butter fat alone. But feed intake was increased so that the rats obtained their necessary calories from the dextrose.

The most complete determination of the lowest level of lactose which will produce diarrhea in weanling rats (Sprague-Dawley strain) was made by Riggs and Beaty (79). They did not observe diarrhea at lactose feeding levels of 5, 10 or 15 per cent, but they did observe diarrhea beginning at the 20 per cent level and increasing in severity and persistence as the level was raised to 30, 40 and 50 per cent. These investigators also showed that the diarrhea was due to the lactose linkage by feeding equivalent levels of the hydrolysis products, glucose and galactose, without producing any diarrhea.

Few reports are available on the amounts of lactose which will produce diarrhea in man, although considerable use was made of the sugar for complementing the action of *Lactobacillus acidophilus* milk in treating constipation. Since it is difficult to tell how much of the action might be due to the lactose and how much to the effects of the bacteria, these reports will be discussed together in a future section, rather than receive consideration under the present heading. In one of the instances in which lactose was fed alone, (that is, without *L. acidophilus* milk) diarrhea was observed by Robinson (80) when 200 to 250 g. of lactose were ingested by human subjects. Koehler *et al.* (50) observed flatuency and diarrhea in only one case, when from 80 to 120 g. of lactose were ingested. Rettger and Cheplin (76, p. 110) found that 300 g. of lactose per day tended to produce diarrhea in man. Kopeloff and Cohen (53) observed that in 6 of 15 subjects the first ingestion of a 100-g. dose of lactose caused diarrhea. Barker (4) recommended adding from 50 g. to 1 lb. of lactose to the diet of typhoid fever patients, as long as there was no diarrhea. He stated that one should remember that

patients given the high-calorie diet he recommended usually have from 2 to 4 stools a day. Traube (96) reported that a light laxative action was produced by only 9 to 15 g. of lactose taken in the morning on an empty stomach. Albertoni (1) stated that 20 g. of lactose were required for action in most cases.

Rojas *et al.* (83) have reported the occurrence of diarrhea in two dairy calves fed separated milk with extra lactose added. The amount of lactose added brought the total lactose content to twice that of separated milk; that is, lactose intake was about 640 g. per day for the Holstein calf and about 500 g. per day for the Jersey calf. The scouring began within a few hours after beginning the feeding of the lactose-enriched separated milk, and decreased when the extra lactose was omitted.

Species differences in susceptibility to lactose diarrhea are indicated in the above accounts of amounts of lactose which produce diarrhea. However, due to the lack of information on actual amounts of the sugar ingested per unit weight, direct comparisons are almost impossible. But in general, it seems that rats are less susceptible to lactose diarrhea than are dogs, while the position of the human in this respect remains obscure.

Age differences also are indicated. Young animals probably are more susceptible to the diarrhea, as shown by Sisson (88) and Ershoff and Deuel (26). Strains of rats also differ in susceptibility, according to Mitchell (60) and Ershoff and Deuel (26). Individual differences have been reported by Gross (36), Sisson (88), and Riggs and Beaty (79).

THE EFFECTS OF LACTOSE ON INTESTINAL MOTILITY IN INFANTS

The importance of lactose in the diet of the human infant is well-known. Hurst (44, pp. 422–424) mentioned that fat and lactose were the two substances in breast milk which stimulated intestinal movements in infants. He called attention to the fact that since undiluted cow's milk has approximately the same per cent of fat, two-thirds as much lactose and more than twice as much protein as human milk, cow's milk usually is diluted for human infants. This dilution makes the protein percentage more nearly comparable to that of breast milk, but at the same time it reduces the percentage of stimulating lactose further below the normal breast milk level. Hurst further commented that the cure of constipation in the non-breast-fed infant often is accomplished immediately by adding 1 teaspoon of lactose to each 3–4 oz. of undiluted cow's milk. For constipation of the breast-fed baby, he recommended 0.5 to 1 teaspoonful of lactose in a little warm water before each nursing.

Talbot and Hill (94) noted that more than 9 to 14 per cent of lactose in the formula resulted in diarrhea in a 5-months-old infant. More than this amount decreased the absorption and perhaps the retention of nitrogen. Porter and Dunn (74) observed that the largest amount of lactose which could be taken by infants without intolerance (meaning without diarrhea, gas and vomiting) varied from 9 to 40 g. per kg. per day, from 2 to 4.75 g. per kg. at a feeding or from 5

to 15.5 per cent of the diet. Paffrath and Siering-Kaulla (69) observed diarrhea and impaired digestion in all trials when 15 per cent lactose solutions were given to children.

Gerstley *et al.* (34) observed that up to 17 per cent lactose added to breast milk produced little change in the fresh weight of infant stools; however, up to 12 per cent added to cow's milk increased the fresh weight and frequency of the stools, but without producing any diarrhea. In a later paper, Gerstley (33) mentioned that a temporary upset in digestion was accompanied by diarrhea for 1 day when an infant was changed to a formula of cow's milk plus 12 per cent lactose. Gerstley's work was begun with the idea that acids produced in the intestine by fermentation of lactose caused the diarrhea observed in some infants. The theory was that lactose fermented more readily when given in cow's milk than when given in breast milk. However, actual results showed that up to 12 per cent of lactose added to cow's milk actually improved the well-being of the infant and did not cause any diarrhea.

Barenberg and Abramson (3) observed somewhat heavier feces (on the fresh weight basis, presumably, although not stated) but no diarrhea, when 12–15 per cent of lactose (15–17 g. per kg. of body weight) was fed to babies 3 to 15 months old.

Skole (91) mentioned that constipation was notably absent in 108 babies (average age 14 weeks at the start) fed evaporated milk and 0.1 oz. of β-lactose per lb. of body weight. Diarrhea sometimes was precipitated when an infant first was started on the diet, but this was controlled readily by reducing the amount of lactose or by omitting it altogether for 2–3 days and then gradually adding it back.

Dennett and Craig (20) observed more diarrhea among breast-fed infants than among infants fed modified fresh cow's milk, evaporated or powdered milk, and more diarrhea on fresh cow's milk than on evaporated or powdered milk. It was not stated whether the powdered milk was dry whole milk or nonfat dry milk solids. The babies were about 2 months old at the start of the experiment.

Jeans and Marriot (46, p. 63) have called attention to the fact that giving lactose solutions of a 10 per cent or greater concentration may result in an increase in the number of stools. Also (46, p. 70) lactose or sucrose solutions of 8 per cent or greater concentration, or dextrose solutions of 6 per cent or greater concentration, are hypertonic and may act as hydragogue cathartics.

Skole (90) claimed, on the basis of case studies, that up to 6 teaspoons of β-lactose per day given with evaporated milk and water shortened diarrheal spells. He offered no explanation of his own, but reviewed the literature for and against the use of lactose in intestinal disturbances of infants. Kendall (47) previously had suggested that adding lactose to cow's milk might decrease the incidence of summer diarrhea of young children. He based this idea on the alteration of the mixed type intestinal flora of the artificially-fed infant's stool to the strictly fermentative type predominant in the normal nursling's stool.

patients given the high-calorie diet he recommended usually have from 2 to 4 stools a day. Traube (96) reported that a light laxative action was produced by only 9 to 15 g. of lactose taken in the morning on an empty stomach. Albertoni (1) stated that 20 g. of lactose were required for action in most cases.

Rojas *et al.* (83) have reported the occurrence of diarrhea in two dairy calves fed separated milk with extra lactose added. The amount of lactose added brought the total lactose content to twice that of separated milk; that is, lactose intake was about 640 g. per day for the Holstein calf and about 500 g. per day for the Jersey calf. The scouring began within a few hours after beginning the feeding of the lactose-enriched separated milk, and decreased when the extra lactose was omitted.

Species differences in susceptibility to lactose diarrhea are indicated in the above accounts of amounts of lactose which produce diarrhea. However, due to the lack of information on actual amounts of the sugar ingested per unit weight, direct comparisons are almost impossible. But in general, it seems that rats are less susceptible to lactose diarrhea than are dogs, while the position of the human in this respect remains obscure.

Age differences also are indicated. Young animals probably are more susceptible to the diarrhea, as shown by Sisson (88) and Ershoff and Deuel (26). Strains of rats also differ in susceptibility, according to Mitchell (60) and Ershoff and Deuel (26). Individual differences have been reported by Gross (36), Sisson (88), and Riggs and Beaty (79).

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SOME EFFECTS OF LACTOSE IN THE DIET OF CHICKENS

Considerable use is made of milk and milk products in poultry feeding. For this reason, the effects of various amounts of milk sugar in the diet of domestic fowl has important practical significance.

Several investigators have fed milk, lactose and milk products to chickens and noted the effects. Shaw (87) concluded from his finding that Barfoed's reagent was not reduced by the intestinal contents of lactose-fed chicks, that lactose was not hydrolyzed in the digestive tract of the fowl. He also observed that chickens fed from hatching on milk alone died after 3 days. Autopsy revealed intense inflammation of gastro-intestinal mucous membranes. Hamilton and Card (38) observed similar irritation of the mucosa when chickens were fed lactose mixed with a moist mash. Diarrhea resulted when over 2 g. of lactose per day were fed. However, up to 8 g. per hen per day were utilized fairly completely, and therefore, the lactose present in the amount of milk, whey or butter milk normally eaten (100–200 ml.) by chickens would be well utilized. It is interesting to note the observation that 8 g. per hen per day represented the approximate upper limit of lactose that the chickens would eat voluntarily under the conditions of their experiment. Plimmer and Rosedale (71), feeding rather high levels of lactose in "seewa" (a dried whey product), did *not* observe any diarrhea.

Beach (5) observed that the pH of the cecal contents of chickens changed from a normal range of pH 6.9 to 7.4 to an acid range of pH 4.4 to 5.6, when he fed lactose alone or in milk products or added to *L. acidophilus* milk. Beach and Davis (6) confirmed this observation of the lowering of cecal pH by lactose. They noted dampness of the litter resulting from watery droppings in the chick pens where lactose or non-fat dry milk solids had been fed. Kline *et al.* (48) also reported both cartharsis and the lowering of the pH of the intestinal content when up to 40 per cent of lactose was fed. The inadvisability of adding 40 per cent of the sugar to rations for day-old chicks was noted. The large amount of fluid material found in the intestine and ceca was stated by Kline *et al.* to be "due undoubtedly to the greater water consumption." Probably this statement is partially true, but there may be other factors contributing to the distention of the ceca and the fluidity of their contents. Asheraft (2) observed marked lowering of cecal pH and some lowering of large intestinal pH by lactose and milk products rations. On such rations the cecal horns were dilated to two or three times the normal size, and contained a yellow, frothy mass of material, while on milk-free rations the cecal content was brown and firm. A ration containing 20 per cent of lactose produced no diarrhea, but rations of dry butter milk, nonfat dry milk solids or dry whey calculated to contain about 20 per cent of lactose produced varying degrees of diarrhea.

THE EFFECTS OF WHEY AND SEPARATED MILK ON GASTRO-INTESTINAL MOTILITY IN MAMMALS

The purgative action of whey probably has been known for a long time. There are some rather recent references to this laxative property for the human,

rat, pig and chicken. Renaud (75) noted slight diarrhea about 2 hours after ingestion of 200 ml. of whey by a fasting human subject. Spindler (93) reported a "copious diarrhea" beginning within a few hours after large amounts (2–8 gallons) of separated milk were fed as the sole food to swine. Frothy feces appeared after about 3 days of diarrhea. In the preceding section, it may be recalled that Ashcraft (2) also noted frothiness of cecal contents of lactose-fed chickens. He also noted more severe diarrhea in chickens fed a dried whey product than in chickens fed other rations containing the same amount of lactose. Fisher (30) obtained preliminary evidence to indicate that lactose was not the sole factor causing diarrhea when whey was fed. On the other hand, the salt content of whey was not the sole factor responsible. These results, while suggestive, are not conclusive, since only a small number of experimental animals was employed. However, the recent work of Daniel and Harvey (19) also indicated that the soluble salts of whey increase the tendency toward diarrhea which exists on lactose rations.

THE MECHANISM OF LACTOSE-INDUCED LAXATION

The mechanism by which lactose acts to produce diarrhea is not definitely known. There are several possible mechanisms:

(a) One possibility is that lactose directly irritates the intestinal musculature, thereby stimulating the muscles and resulting in stronger or more frequent contractions. Results obtained by Feldberg and Solandt (29), with isolated intestinal segments from rabbits, indicate that this is not the case. More evidence supporting this observation should be obtained. Rettger and Cheplin (76) noted that when enough lactose (300 g.) was ingested, diarrhea tended to occur and much lactose was excreted in the feces. From this observation they concluded (76, p. 110) that "the excess of lactose in itself caused some intestinal irritation either directly or through the production of more acid than could be neutralized or absorbed as quickly as it is formed."

(b) A second possible mode of action of lactose in producing laxation is that of a hydragogue, that is, a substance which produces a watery purgation. Presumably, hydragogues are effective because, being only slowly absorbed from the intestine, many of their ions or molecules remain within the lumen of the gut and thus raise the osmotic pressure therein above that of the blood. Therefore, water diffuses into the intestinal lumen from the blood. This excess water distends the intestinal walls, thus stimulating muscular contraction and also flushes the intestinal tract. Lactose seems to fit this description of a hydragogue for the following reasons. First, it produces watery purgation. Second, it is absorbed[2] at only one-third to one-half the rate of dextrose, maltose or sucrose (1), because it is more slowly broken down (97, p. 115). Some lactose may escape digestion in the small intestine and pass into the large intestine (97, p. 114; 76, p. 60, p. 107). The presence of lactose molecules in the intestinal lumen thus could exert

[2] The term "lactose absorption" has been used in this review to mean hydrolysis and absorption as the monosaccharides, glucose and galactose (unless otherwise stated). Most lactose hydrolysis probably occurs during the process of absorption (31).

the supposed hydragogue action. In support of the possibility that lactose acts in this way is the work of Albertoni (1) in which large dogs, fasted for 48 hours, were given from 67 to 167 g. of lactose in solution. The dogs were killed an hour later, and it was found that considerable lactose and water remained in the intestine, and that the stomach and intestine together contained more liquid than had been given.

In the case of such a hydragogue action, if water passes from the blood into the intestinal lumen, then either dehydration of body tissues or decreased urine volume might be expected. That the action of lactose in producing diarrhea may be due to such a dehydrating action was suggested by Whittier (99). Jarvis (45), commenting on his observations of babies and on Killian's unpublished work with rats, thought that lactose diarrhea might be one cause of the lowered water content of rat tissues which resulted when lactose was fed. In Killian's work lactose was compared with sucrose. The data reported by Whittier *et al.* do not entirely support the work of Killian. From table 2 in the paper by Whittier *et al.* (101) it can be calculated that the percentage moisture of the net body weight was greater in the lactose-fed rat than in the sucrose-fed rat of each pair, with *ad libitum* feeding. However, in two pairs of pair-fed rats killed and analyzed at 774 days of age, this difference in tissue moisture was not evident, as can be calculated from table 4 of the same paper. Mitchell *et al.* (56) found more water in the tissues of rats fed lactose, as compared to tissues of rats fed glucose. Handler (40), judging from studies of hematocrit values and serum protein content of the blood of rats fed 81 per cent lactose rations, found no serious dehydration occurring. Urine volume was three times as great as that of a comparable group of rats fed an 81 per cent sucrose ration. This greater urine volume was the result of excretion of galactose in the urine. The data presented by Mitchell and Isbell (57) show that the volume of urine produced by rats on a ration containing 75 per cent ground beef and 25 per cent lactose is not different from that produced by rats on a 100 per cent ground beef ration. Roccuzzo (82) found that *less* urine was eliminated by men after taking lactose solutions (hypertonic, hypotonic or isotonic solutions) orally than after taking an equal volume of water. Holt and Kajdi (41) found that feeding rats lactose as the sole article of diet resulted in a greater urine volume, diarrhea and a greater water intake. On the other hand, Ershoff (25) did not report a greater water intake by rats fed lactose as the only food. However, greater water intakes have been reported by others when 87.5 per cent lactose rations were fed to rats (35), and when 40 per cent lactose rations were fed to day-old chicks (48).

Two groups of investigators, Schorer and Laffoon (84) and Epstein and Thompson (24), presented evidence that lactose-sodium citrate solutions were effective in diminishing the so-called "dehydration" weight loss of newborn infants. The sodium citrate was supplied as a hydrating agent, the lactose as a food. The β-lactose-sodium citrate solution was more effective than the α-lactose-sodium citrate solution in this respect. Also, the β-lactose solution alone was

more effective than the α-lactose solution, but neither sugar alone was as effective as either of the lactose-sodium citrate combinations. This evidence seems to indicate that the sodium citrate was the main hydrating agent involved, while the lactose effect on birth weight decline may be due to its value as a hydrating agent, or as an energy source. The hydrating or dehydrating action of lactose therefore is not yet definitely established.

Hurst (44 p. 331) was of the opinion that sweet milk had practically no effects on bowel movements, although many persons considered milk to be constipating. Much more recently, Hosoi *et al.* (42) have stated that milk should not be given if it is desired to prevent bowel movements, because milk leaves a large residue in the terminal ileum. Their experiments, which were performed with dogs, indicate that when a small residue is desired, any common food sugar *except* lactose may be included in the low residue diet. Milk and lactose were predominant among the foods which left both a large moist residue and a large dry residue. This copious dry residue suggested to these investigators also that lactose, due to poor absorption by the mucosa, might act by increasing the fluid content of the intestine and thus produce laxation in the same manner as does magnesium sulfate. A solution having a lactose concentration equal to that of milk passed very rapidly through the small intestine. But, in general, when lactose was fed with other foods, the rate of passage of the residues through the small intestine was not increased, while the total residue excreted was increased. Childrey *et al.* (15) later reported that lactose given 1 day interfered with the digestion of foods eaten both on the same day and on the following day. Mitchell *et al.* (56) have also noted the impaired digestion of organic nutrients resulting from lactose feeding. Outhouse *et al.* (68) showed that the fecal pellets of lactose-fed rats had a significantly greater dry weight than the fecal pellets of litter mates fed different rations. Mitchell and Isbell (57) also found that heavier feces (on the wet basis) were obtained from lactose-fed rats. Other references to the effects of lactose on the digestibility of organic nutrients and on the amount of feces excreted have been discussed in the section on intestinal motility in infants. The reported evidence thus seems to show rather conclusively that lactose *does* increase the amount of food residue excreted from the intestine.

Koehler *et al.* (50) have suggested that *if* the failure of lactose (1.5 g. per kg.) to raise the blood sugar level of normal young adults is due to delayed absorption resulting from slow hydrolysis of the lactose, then the rate of hydrolysis of lactose must be very much slower than that of starch. They have suggested further that rapid passage of lactose residues through the intestine may interfere with hydrolysis, even when appreciable amounts of the lactose-splitting enzyme are present, but no experiments were performed to test this possibility. The question of whether the fast rate of passage of lactose is the cause or the effect of the slight hydrolysis of this sugar thus remains to be settled.

Further evidence that the amounts of lactose hydrolyzed are small is furnished by many observations of "pot-bellies" in lactose-fed animals. Mitchell (60) described this pot-bellied appearance in rats of all strains used by her, when 70

per cent lactose rations were fed. Autopsies revealed that the cecum usually was greatly distended and filled with fluid or a semi-solid mass of material. Mitchell noted that the Wistar strain suffered greater cecal distention than the other strains, due mostly to more gas. She reasoned that rats of this strain probably did not absorb as much of the lactose as rats of the other strains. The bloated condition also has been observed by Mitchell *et al.* (56). Other investigators (54, 63, 95) have observed that the ceca of lactose-fed rats were distended and contained a watery, whitish material, which they presumed to be mainly unabsorbed lactose. Ershoff and Deuel (26) reported that rats fed 73.2 per cent lactose diets sometimes had ceca enlarged in size to almost 20 times those of similar rats fed non-lactose rations. Ershoff (25) found that all of his rats fed only lactose had pot-bellies when alive and showed distention of the cecum at autopsy. Riggs and Beaty (79) observed abdominal distention of increasing persistence and severity as the percentage of lactose in the ration was raised from 20 to 25, 30, 40 and 50 per cent. Asheraft (2) noted that the ceca of lactose-fed chickens were distended two to three times the normal size. It can be seen in table I of the paper by Mitchell and Isbell (57) that the actual wet weight of the cecal content was more than doubled by incorporating 25 per cent of lactose into an all-meat ration. Similarly, table 2 in the paper by Whittier *et al.* (101) shows a heavier intestinal content as a result of lactose feeding.

(c) Possibly lactose acts to produce diarrhea indirectly through the alteration of the intestinal flora to an aciduric type. This aciduric flora might then produce diarrhea in any one or more of several possible ways. That the flora alteration is not the primary cause of the diarrhea is indicated by the rapid onset of the diarrhea after lactose feeding (26), while the complete flora transformation requires from 3 to 6 days (76, p. 17, p. 76).

(d) Another way that lactose may act is by changing the pH of the intestinal contents enough to stimulate the intestinal muscles directly. Robinson (80) has shown that laxatives, including lactose, produce a lowered pH in the *feces* of men. Others (2, 5, 6, 14, 43, 48) have demonstrated a lowered pH in the intestinal contents of various species when lactose was fed. Robinson and Duncan (81) reported that the degree of pH lowering by lactose was dependent upon the character of the diet. A much greater effect was produced by a vegetable diet than by a meat diet, even though each diet contained the same percentage (25 per cent) of lactose. The possible relationship of the change in intestinal flora to the change in acidity has been investigated extensively (14, 43, 51, 73, 76, 98). However, Beach (5) has shown that, in the chicken at least, the initial pH change occurred very quickly (2 to 2.5 hours) after lactose feeding before much change in the flora could have occurred. Therefore, the *initial* pH change associated with lactose ingestion probably is not the result of the flora change. It is possible that the pH change is responsible for the laxation. However, it seems more probable that the laxation is responsible for the initial pH change, for the increased rate of passage of residues would be expected to interfere with the neutralization and absorption of acids (37).

(e) Bergeim *et al.* (7) have shown that lactose maintains a positive oxidation-reduction potential in the intestine. Of the other foods tested, only raw apple possesses in common with lactose the ability to maintain a positive potential. The possible relation of this observation to lactose diarrhea remains obscure, but it is interesting to note that raw apple is considered by many to be a good bowel regulator.

(f) There is one more conceivable way in which lactose may produce its laxative effects. It has been shown repeatedly that the laxative action is due to the lactose molecule *per se*, and not to its hydrolysis products. When a large amount of lactose is ingested, a small part of the disaccharide may be absorbed into the blood stream without being hydrolyzed in the process (97, p. 115). Such minute amounts of unsplit lactose conceivably may act from the blood on the nerve supply of the intestine to increase muscular activity. Such a theory has been proposed (44, pp. 349–350) for the mode of action of saline purgatives. However, this possibility seems unlikely in the case of lactose, since diarrhea apparently has not been reported after lactose injections into the blood stream, but perhaps no observations were made to determine whether or not diarrhea had occurred following the lactose injections. Furthermore, in the case of *injected* lactose, the rapid renal elimination of the sugar would be expected to prevent a prolonged stimulating effect, while in the case of *ingested* lactose, the continuous absorption of small amounts of the unsplit lactose molecule might be expected to exert a prolonged action.

SOME EFFECTS OF OTHER DIETARY CONSTITUENTS ON LACTOSE-INDUCED DIARRHEA

The work of several investigators indicates that the kind and amount of salts ingested with lactose affects the rate of lactose hydrolysis and the incidence and severity of diarrhea. Mitchell *et al.* (61) fed rats adequate diets containing 60 per cent lactose and the Osborne and Mendel salt mixture. This mineral mixture supplied 0.5 per cent calcium. Additional calcium (0.5 or 1 per cent) was added in the form of a different salt for each experiment. The phosphate, carbonate, citrate and levulinate of calcium at both levels gave good growth, the same degree of galactemia and incidence of galactose cataract, *but less diarrhea, in general,* than the 60 per cent lactose ration without added salts. Calcium lactate at the 0.5 per cent level gave similar results, but at the 1 per cent level it tended to decrease diarrhea, yet produced toxic effects. Both the 0.5 and the 1 per cent levels of calcium gluconate gave different results. Poor survival, toxic symptoms, weight loss, low blood sugar and *no* galactose cataract were observed together with diarrhea of a severe nature, especially when the 1 per cent level of calcium gluconate was fed. Sodium gluconate gave similar but more severe results than calcium gluconate. Calcium gluconate apparently did not inhibit the absorption of glucose or galactose or the digestion and absorption of sucrose, dextrin or starch. Growth efficiency was as good with gluconate as without, when glucose and galactose were fed. Therefore, it was thought that the gluconate radical somehow interfered with lactose utilization, perhaps by inhibiting lactase

enzyme activity. Most of the rats fed the lactose ration plus calcium or sodium gluconate showed as severe diarrhea after several weeks as at first; thus adaptation generally was prevented by the gluconates. There were a few rats which survived the feeding of lactose plus gluconate, showing improved growth rate and efficiency of gain in spite of the persistent diarrhea. In these inexplicable cases, the growth recovery indicates the occurrence of adaptation while the continuing diarrhea contraindicates such an occurrence.

Coryell and Christman (17) have presented evidence to show that calcium chloride given with lactose by stomach tube to fasted rats decreased the hydrolysis of the lactose and also decreased the absorption of the galactose. It has been shown by Nordbö (66) that lactose combines with calcium and magnesium to form non-ionized compounds. Nalder (64) thought that this combination might explain the interference of calcium with lactose hydrolysis and galactose absorption.

Riggs and Beaty (79) found no difference in growth, food utilization or occurrence of diarrhea when litter-mate rats were fed a 50 per cent lactose diet containing 3.5 or 7 per cent of a salt mixture (U.S.P. XII salt mixture #2).

The salts of whey, as compared to an equal percentage (13 per cent) of the salts of McCollum's salt mixture, were found by Fisher (30) to intensify diarrhea on a 41 per cent lactose ration. Daniel and Harvey (19) reported that 50 per cent lactose rations containing whey protein produced a more persistent diarrheal condition when the soluble ash of the whey remained with the protein than when this ash was removed by dialysis.

Boutwell *et al.* (10) reported poor outward appearance and the occurrence of diarrhea in rats during the first 6 to 12 days of feeding a ration containing 48 per cent lactose and 28 per cent corn oil. A similar ration containing 28 per cent butter fat in place of the corn oil gave better growth, larger feed intake, and good outward appearance. Boutwell *et al.* (11) later reported less diarrhea among rats fed the ration containing 48 per cent lactose and 28 per cent butter fat than among rats fed the ration containing 48 per cent lactose and 28 per cent corn oil. On the other hand, Deuel *et al.* (21) reported no difference in nutritive value of butter fat and vegetable fats when fed to rats in a ration with 70.6 per cent of nonfat dry milk solids and vitamin supplements. These workers eliminated from the experiment any litter of which two or more members developed diarrhea. It would appear that if butter fat prevents the occurrence or reduces the severity of lactose-induced diarrhea, then a physiological difference between the food fats in question has been demonstrated. Perhaps this point should be considered more carefully in work of this sort. In a later experiment, Ershoff and Deuel (27) studied the length of survival of rats fed diets of 70 per cent carbohydrate and 30 per cent fat. Survival was longer with sucrose than with lactose. The kind of fat fed did not influence significantly the length of survival on the sucrose rations, but on the lactose rations butter fat or margarine allowed significantly longer survival than did corn oil or cottonseed oil.

Rojas *et al.* (83) found that the absence of fat from the diets of dairy calves did not interfere with the utilization of the amounts of lactose usually consumed

in a separated milk ration. As noted previously, doubling the amount of lactose in the separated milk produced diarrhea. However, no study was made of the effect of doubling the lactose content of *whole* milk fed to calves.

Ershoff and Deuel (26) observed severe diarrhea and death of weanling rats fed a 73.2 per cent lactose ration. Each rat was given 800 mg. of corn oil per day. The average survival time for rats of the Long-Evans strain fed β-lactose was 4.9 days. When 10 per cent of lard, oleo or butter fat was fed, the average survival times were 2.4, 3.0 and 3.2 days, respectively. Mitchell (59) fed rats rations containing *18 per cent of butter fat* and varying amounts of lactose. At the 60 and 45 per cent lactose levels, growth was poor, while at the 30 per cent level it was practically normal. However, Riggs and Beaty (79) reported slower growth in the early part of an experiment in which rats were fed diets containing 5 per cent fat and 30 per cent lactose. These rats recovered and weighed as much after 12 weeks as rats fed the basal cornstarch ration. That the difference in growth at the 30 per cent lactose level was perhaps due to the higher fat content of Mitchell's diets was suggested by Riggs and Beaty. Their observation of rapid growth and only slight diarrhea in rats fed dried whole milk (27.5 per cent fat, 37.5 per cent lactose) where the lactose intake was about 33 to 34 g. per kg. per day supports this suggestion. Such large lactose intakes caused severe diarrhea on the 5 per cent fat ration. Thus apparently the favorable effect of larger amounts of fat was due not to a reduction in lactose intake, but rather to an improvement in lactose utilization. However, Riggs and Beaty (79) did not think it likely that fat directly affected the hydrolysis of lactose. Such a conclusion is supported by the work of Coryell and Christman (17). They showed that vegetable fat (Mazola) given with lactose by stomach tube did not alter significantly the amount of lactose hydrolysis or of galactose absorption. Nieft and Deuel (65) also reported that corn oil did not alter lactose hydrolysis, but contrary to the results of Coryell and Christman (17), they reported that galactose absorption was delayed by fat. These contradictory results as regards galactose absorption perhaps may be explained by differences in the methods used.

Ershoff and Deuel (26) tried adding various supplements to the 73.2 per cent β-lactose diet which they fed to weanling rats of the Long-Evans strain. The average survival time for such rats fed the β-lactose ration without supplements was 4.9 days. The survival time was lengthened to 7 ± 2 days by feeding 10 per cent of dried mammalian liver, and to 11 ± 3 days by feeding dried tuna liver. The rats fed these supplements gained weight the first 5 to 7 days, then lost weight and showed the typical β-lactose syndrome, including severe diarrhea. Other supplements (lard, oleo, butter fat, cream, wheat germ oil, biotin, brewers' yeast) were ineffective in lengthening survival time.

THE USE OF LACTOSE WITH AND WITHOUT L. ACIDOPHILUS FOR TREATING CONSTIPATION AND TRANSFORMING THE INTESTINAL FLORA

Lactose has been used extensively in conjunction with *L. acidophilus* milk for treating constipation of human adults, making difficult any evaluation of the

effect of the sugar alone. However, a few investigators have employed the sugar alone. Traube (96) recommended 9 to 15 g. of lactose in 0.25 l. of warm milk, taken in the morning on an empty stomach. Albertoni (1) recommended 20 g. of lactose taken in water. Boros (8, 9) reported that 16 out of 20 cases of constipation selected at random were benefited by lactose therapy and a prescribed diet. Two tablespoons of lactose in one-half glass of cold water were given twice a day. This is equivalent to about 38 g. per day.[3] In some cases a few days were required before results were observed. The doses of sugar could be reduced or cut out entirely after normal bowel habits were established.

Metchnikoff (55) first recommended the use of Bulgarian sour milk as a means of prolonging life, reasoning from observations of the long life of some Bulgarian peasants and from some rather meager experimental results obtained in his laboratory. His theory was that *Lactobacillus bulgaricus* became implanted in the intestine to the exclusion of harmful bacteria. However, several investigators have shown that *L. bulgaricus* does not survive in the digestive tract.

Rettger and Cheplin (76) have given a complete review of the literature on the subject of sour milk organisms in the intestine and have given a detailed account of their work in the field. They showed that *L. acidophilus* and *L. bifidus* (which may be identical organisms) can be stimulated to grow and to form a large part of the intestinal flora by feeding lactose, dextrin or massive doses of cultures of the organism. The probable explanation of the stimulating action of lactose and dextrin alone among the carbohydrates is that only these two carbohydrates reach the large intestine in large amounts and there provide a suitable food source for the *L. acidophilus* organism. These workers found that the intestinal flora of rats usually was transformed completely to an aciduric type by feeding 2 g. of lactose or dextrin per day, by 2 ml. of a culture of *L. acidophilus* or by 1 g. lactose or dextrin with 1 ml. of the culture. In a similar manner, the human intestinal flora usually was transformed completely by 300 to 400 g. of lactose or dextrin, by 300 ml. of *L. acidophilus* culture or by 150 g. of lactose or dextrin and 150 ml. of the culture. Also, in man, the flora transformation was effected by 1000 ml. of a 2-to-24 hour culture of *L. acidophilus* in milk or by 500 ml. of such a milk culture plus 100 g. of lactose.

It is interesting to note that 300 g. of lactose, the dose usually needed for complete transformation of the flora in man, corresponds to the approximate level where a tendency toward diarrhea was observed by these investigators. Others (1, 53, 80, 96), however, have observed diarrhea in man after ingestion of somewhat smaller amounts of lactose. Rettger and Cheplin were aware of a possible direct effect of lactose on intestinal motility. However, Rettger *et al.* (77) considered that the relatively large amounts of lactose which they employed with *L. acidophilus* milk for successfully treating some types of constipation constituted a pabulum for the organisms, rather than having any direct benefit.

[3] This figure of 38 g. per day was obtained by assuming that one level tablespoon of lactose weighs ½ oz. Thus the four-tablespoon intake would correspond to 1⅓ oz., or 37.8 g.

Kopeloff and Cheney (52) obtained relief from chronic constipation by giving 1000 ml. of *L. acidophilus* whole milk and 300 g. of lactose per day. They also treated diarrhea with the milk, but omitted the lactose because of its known diarrheal effects. Kopeloff (51, p. 109) satisfied himself by numerous experiments that the effects of the *L. acidophilus* milk were mainly bacteriological, rather than physical or chemical. He (51, p. 52) realized the benefits in treating constipation, of using lactose with the milk and of continuing the use of lactose after discontinuing the milk treatment for prolonging the beneficial effects. He postulated (51, p. 53) that the benefits might be due either to lactose being a suitable pabulum for *L. acidophilus* in the intestine, or to lactose *per se* relieving constipation. Apparently, he believed the first possibility to be the case, since the use of lactose alone did not produce as uniformly good results as its use with *L. acidophilus* milk, and the addition of lactose to *L. acidophilus* milk produced no great improvement over the use of *L. acidophilus* milk alone.

On the other hand, Clark and Perry (16) got much better results when lactose was given with *L. acidophilus* milk than when the milk was given alone. They reported less constipation in 80 per cent of their patients given 1 quart of *L. acidophilus* milk per day with 75 g. of additional lactose, while only 30 per cent of the patients given 1 quart of the milk alone were less constipated, and 40 per cent were more constipated. It is to be noted that these workers used much smaller amounts of lactose than did Rettger *et al.* (77) and Kopeloff (51). A possible explanation for the failure of Clark and Perry to get better results with the milk alone might be that their milk perhaps did not contain sufficient numbers of viable organisms. Cruickshank (18) thought that the effectiveness of *L. acidophilus* milk was due to a combination of viable organisms and the unfermented lactose in the milk.

The results of these various investigators seem to conflict. It is difficult to ascertain from a study of their work whether or not lactose alone has any therapeutic value in treating constipation, but the indications are that it possibly has some such value. It does seem, from a consideration of the work which has been done, that lactose has at least indirect benefits in constipation through the encouragement of the *L. acidophilus* organisms. According to Kopeloff (51, pp. 115–116) these organisms may produce their benefits in several possible ways:

"(1) *L. acidophilus* may secrete enzymes which act directly upon the intestinal mucosa to stimulate peristalsis. (2) *L. acidophilus* may secrete or excrete substances toxic to putrefactive organisms. (3) *L. acidophilus* may change the hydrogen ion concentration of the intestinal tract to such a degree as to inhibit the growth of putrefactive organisms. (4) *L. acidophilus* may change the hydrogen ion concentration of the intestinal tract to such a degree as to stimulate peristalsis directly. (5) *L. acidophilus* may produce lactic acid of chemical structure different from inactive lactic acid or that produced by other microorganisms usually present in the intestine which may directly stimulate peristalsis."

It has been suggested by Smith (92) that a high-lactose diet alone is not so effective in transforming the flora in the intestine of man as in the intestine of

the rat. This relative ineffectiveness of lactose alone partially may explain the variability of results which have been obtained in attempted therapeutic applications of *L. acidophilus* and lactose feeding in man. Smith (92) also observed that the transformation of the intestinal flora was aided by giving laxatives with lactose. He reasoned that the laxatives probably increased the rate of passsage and hindered absorption of the lactose, allowing larger amounts to reach the lower bowel, there to stimulate the aciduric organisms. This observation confirms the theory of the Yale workers (76) that lactose can stimulate *L. acidophilus* because such large amounts reach the lower bowel unabsorbed.

Mitchell (59), working with rats, observed that increasing percentages of food sugar lost in the feces and increasing percentages of *L. acidophilus* paralleled increasing percentages of lactose in the ration. *L. acidophilus* constituted 50 per cent or more of the flora when 30 per cent or more of lactose was fed. But at 45 and 60 per cent levels of lactose feeding, poor growth of the rats was obtained, even though a higher percentage of *L. acidophilus* was found in the flora. In a companion paper, Mitchell (58) found that the high percentage of *L. acidophilus* lasted only while diarrhea accompanied lactose feeding. On a 60 per cent lactose ration, the *L. acidophilus* count dropped from 80–95 per cent of the total flora to 13–15 per cent of the flora when intestinal stasis was produced by giving a few grains of "tannalbin" daily as an astringent. The diarrhea stopped in a few days and the growth rate changed from a decreasing rate to an increasing rate in 2 weeks. When Dragstedt *et al.* (22) produced intestinal stasis by reversing a 12-inch segment of intestine or by ligation of the intestine, the flora was proteolytic in character, irrespective of the nature of the diet.

ADAPTATION TO LACTOSE FEEDING

An animal may adapt to lactose feeding; that is, the diarrhea may become less severe, may occur less commonly or may even cease entirely if the animal continues to ingest rather large amounts of lactose over a period of time. Several investigators have reported adaptation to lactose feeding in rat experiments. Whittier *et al.* (101) noted that rations containing 45 and 63.5 per cent of lactose produced diarrhea and retarded growth in weanling rats, but that these rats recovered in about 3 weeks. Mitchell and Dodge (62) observed that the diarrheal condition of young rats fed 70 per cent lactose diets subsided in time, but the fecal pellets continued to be softer than usual. Morgan *et al.* (63) reported cessation of diarrhea after a week or two in rats fed a 65 per cent lactose ration. Mitchell *et al.* (61) found that young rats fed rations containing 50 per cent or more of lactose outgrew the lactose diarrhea in a few weeks, except when the ration contained 0.5 per cent of calcium gluconate or of sodium gluconate. When either of these salts was included in the diet, the severe diarrhea in most cases persisted after several weeks. These investigators implicated the gluconate radical; and since this radical did *not* inhibit glucose or galactose absorption, they thought that it must interfere with lactose hydrolysis, possibly by some mechanism of "competitive inhibition" of lactase action. Riggs and Beaty (79)

recently have shown again that rats, through the repeated daily ingestion of lactose, develop the ability to utilize lactose without suffering from diarrhea.

Possibly the mechanism of adaptation to lactose feeding is an increased production of the lactase enzyme in the intestinal mucosa, but there is no evidence in the literature to support this possibility. Mitchell (58) noted that the aciduric intestinal flora produced by lactose feeding tended to revert to the putrefactive type after a time if lactose ingestion was continued. This observation may indicate that smaller amounts of unhydrolyzed lactose were reaching the lower levels of the bowel, and therefore that lactose-hydrolyzing ability had been increased in some way. One possibility is that the mucosal cells lining the intestine had been stimulated to produce larger amounts of lactase enzyme. The previously cited observations of decreased incidence and severity of lactose-produced diarrhea after some time on lactose diets may indicate the same mechanism of increased lactose hydrolysis due possibly to increased lactase production. Therefore, a consideration of the properties and occurrence of this enzyme is important.

Florey *et al.* (31) reviewed some of the literature on the subject of lactase and concluded that most of the lactase action is due to the mucosal cells rather than to the intestinal juice, and therefore, that most lactose digestion occurs during absorption.

Plimmer (70) used a copper reduction method to show that lactase was present in the intestines of young mammals and persisted in older mammals of some species, but not of other species. The guinea pig, for example, lost its intestinal lactase activity in 5 weeks after birth, while lactase activity continued to some extent in the adult dog and rabbit. Experiments showed that lactase production was not increased by feeding milk and lactose.

Plimmer and Rosedale (72) and Hamilton and Mitchell (39) demonstrated the presence of the enzyme in the crop of the chicken, but not in the proventriculus, pancreas or intestine.

Cajori (12) could not find lactase in intestinal juice and thought that either lactose must be absorbed in part without being hydrolyzed, or else the enzyme action was carried on in the mucosal cells. Later he (13) reported lactase in the jejunal and duodenal mucosa and in the liver. The jejunal mucosa had a lactase activity 10–30 per cent greater than that of the duodenal mucosa. The liver lactase activity was very small. The optimum pH range for dog intestinal lactase was 5.4 to 6.0. Glucose inhibited lactase action, for in the presence of glucose 40 per cent less lactose was hydrolyzed in 22 hours. Galactose had practically no effect.

Ershoff and Deuel (26) reported a strain difference with respect to average survival time and severity of diarrhea in rats fed a 73.2 per cent lactose ration. They proposed that this difference might be due to a difference in intestinal lactase activity. The University of Southern California strain was not as severely affected as the Long-Evans strain, indicating possibly greater lactose hydrolysis. Species, age and individual differences in susceptibility to lactose feeding have

been discussed previously, and these also may be due to differences in lactase activity.

Koehler *et al.* (50) expressed doubt that slight lactose hydrolysis necessarily was the cause of the rapid rate of passage of lactose. They theorized that the rapid passage might be the cause of the slight hydrolysis, even when considerable amounts of lactase were present, since there would be little time for enzyme action. If this were so, then perhaps some method of action of lactose other than a hydragogue effect would be indicated.

SUMMARY

The literature dealing with the effects of lactose on the rate of movement of food residues in the gastro-intestinal tract has been reviewed. Species, strain, age and individual differences in susceptibility to lactose-induced laxation have been reported.

A lactose feeding level of 20 per cent of the diet often induces diarrhea in weanling rats of the Sprague-Dawley strain. When lactose in sufficient amounts to produce diarrhea is fed, the animals exhibit pot-bellies, slow growth and reduced feed consumption. These symptoms become progressively more severe and persistent as the percentage of lactose in the ration is increased. The lowest levels of lactose feeding which will initiate diarrhea in species other than the rat have not been carefully determined, although the occurrence of diarrhea has been reported in all species studied.

Early death in rats fed rations containing 61.8 to 100 per cent lactose has been reported by several investigators.

The lactose linkage apparently is responsible for the diarrhea and some of the other symptoms noted when moderate to large amounts of lactose are fed.

The literature reports, based generally upon case studies rather than upon carefully controlled experiments, indicate that more than about 12 to 19 per cent of lactose in the formula is apt to cause diarrhea in human infants. This may be compared with 10 to 15 per cent for puppies, and 20 per cent for weanling rats. Other reports suggest that smaller amounts of lactose may be valuable in controlling or preventing diarrheal conditions.

The available evidence indicates that lactose in large amounts (20 per cent or more of the ration) tends to produce diarrhea in chickens and to lower the pH of the contents of the ceca markedly and of the large intestine somewhat less. The ceca are distended by a frothy liquid material. Water consumption is increased. When lactose constitutes about 40 per cent of the ration of young chicks, extreme inflammation of intestinal mucous membranes may result, and death often may ensue.

The evidence seems to show that the purgative action of large amounts of skim milk and whey is due partly, but not entirely, to lactose.

Lactose may act to produce diarrhea in several possible ways, for example:

(a) Direct stimulation of intestinal muscles. (b) Hydragogue action; interfering with absorption of water and organic nutrients. (c) Alteration of intes-

tinal flora to an aciduric type, the aciduric flora then acting in several different possible ways. (d) Alteration of pH enough to stimulate the intestinal musculature. (e) Maintenance of a positive oxidation-reduction potential. (f) Stimulation of intestinal muscle through its nerve supply by small amounts of unhydrolyzed lactose in the blood.

Of these possibilities, the first, third and sixth are contraindicated by the available evidence, while the fourth appears to be an effect rather than a cause of the diarrhea. The possible relation, if any, of the positive oxidation-reduction potential to lactose diarrhea is not clear. Thus, at the present time, the second possibility, that of a hydragogue type of action, seems most plausible. However, some evidence does not support the idea that tissue dehydration is accomplished by persistent lactose diarrhea. Dehydration would be expected if a continuous hydragogue action were involved, unless water consumption were increased or urine volume decreased. It has been shown that water intake sometimes is increased, at least on rations consisting solely or largely of lactose. On such high-lactose rations, urine volume also increases as a result of the urinary excretion of galactose, whereas urine volume decreases in many diarrheal conditions. However, the work of one investigator does indicate *decreased* diuresis when lactose is given. Water balance experiments at lower lactose feeding levels seem to be desirable in order to better evaluate the possibility of a hydragogue type of action.

Other dietary constituents seem to influence the occurrence, severity and persistence of lactose-produced diarrhea. Certain calcium salts may decrease the severity of the diarrhea to some extent, while both calcium gluconate and sodium gluconate seem to increase the severity of the diarrhea and prevent adaptation to lactose feeding. It has been demonstrated that calcium chloride decreases the rate of lactose hydrolysis and galactose absorption. A certain portion of the salts of whey intensifies and prolongs lactose diarrhea. Increasing the amounts of fat in the diet tends to decrease the diarrhea, and there is some indication that butter fat may be more valuable in this respect than certain other food fats. The feeding of liver may postpone but not prevent the appearance of lactose-feeding symptoms on a high-lactose diet.

Lactose has been used successfully in conjunction with and for prolonging the effect of *L. acidophilus* in transforming the intestinal flora to a predominantly aciduric type. The use of lactose without *L. acidophilus* seems to have definite therapeutic possibilities as yet little investigated. Evidence indicates that the permanent transformation of the flora by lactose depends upon sufficient amounts of the sugar continually arriving in the lower bowel, for the flora reverts to the normal mixed type when lactose feeding is discontinued or when intestinal stasis is produced.

Adaptation to lactose feeding is evidenced by the diarrheal condition becoming less conspicuous or disappearing completely.

Lactase occurs in the mucosal cells of the intestines of mammals, but not to any extent in the intestinal juice. Therefore, lactose hydrolysis must be mainly an

intracellular process, occurring during absorption. Since lactose diarrhea probably is the result of slight lactose hydrolysis and the resulting hydragogue effect, then the species, strain, age and individual differences in the severity of lactose feeding symptoms might be due to differences in intestinal lactase activity. Also, it seems likely that adaptation to lactose feeding is brought about by increased intestinal lactase activity. The results of one early investigator indicate that increased lactase activity does *not* result from continued milk or lactose feeding, but these experiments have not been confirmed.

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ASCORBIC ACID OXIDATION IN MILK BY PREFORMED HYDROGEN PEROXIDE

VLADIMIR N. KRUKOVSKY

Department of Dairy Industry
Cornell University, Ithaca, N. Y.

The following experiments were undertaken to ascertain whether the activity of peroxidase or of some other substances were responsible for the rapid promotion of oxidation of ascorbic acid in milk to dehydroascorbic acid by preformed H_2O_2. The preliminary information pertaining to the subject was obtained in connection with the studies of the mechanism of the reactions involving ascorbic acid oxidation by added H_2O_2, resulting in the stabilization of the lipid component of the milk system (4, 5). It was observed that the rate of ascorbic acid oxidation varied inversely with the volume of 30 per cent H_2O_2 added to milk, and the reaction was retarded appreciably when the amount of the reagent added was in excess of that required to oxidize ascorbic acid completely (5). From 0.021 to 0.03 ml. of 30 per cent H_2O_2 usually was needed to complete ascorbic acid oxidation in the milk. This retardation of ascorbic acid oxidation suggested that the reaction is catalyzed by an enzyme which in turn is slowly inactivated by the H_2O_2. It was of interest, therefore, to learn if the heat inactivation of peroxidase in milk would result in non-reactivity of ascorbic acid and H_2O_2, and if the ability of milk to utilize H_2O_2 for the oxidation of ascorbic acid could be restored by the addition of plant peroxidase.

The experimental conditions adopted for this study also were found to be convenient for the reinvestigation of the copper catalysis of ascorbic acid oxidation by H_2O_2. This approach was prompted by the studies of Hand and Chase (3), which indicated that the differences between copper and light in their effects on the oxygen combining power of vitamin C could be accounted for by the assumption that copper catalyzes the oxidation of ascorbic acid by H_2O_2.

EXPERIMENTAL

Zilva (8), in his work on the rate of heat inactivation of peroxidase in milk, has shown that heating for several hours at 65° C. produced no effect on the peroxidase activity of milk, and that 1.3 minutes at 75° C. reduced its activity to 10 per cent. Consequently, the samples of milk used in the present study were heated for 30 minutes at 61.1 and 76.6° C., respectively. Portions of these samples then were treated with either one or several reagents in the following order (per liter of milk): 0.09 ml. of H_2O_2 (10 per cent, standardized by titration with potassium permanganate), 1 ml. of horseradish peroxidase solution (1) and 0.1 mg. of copper (as copper sulphate). They were held at 20° C. during the first hour of digestion and then at 2 to 5° C. throughout the duration of the experiment. The changes in the ascorbic acid content of

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the samples were followed by direct titration with 2,6-dichlorophenolindophenol in acid solution (6).

A comparison of data presented in figure 1 revealed that ascorbic acid was oxidized rapidly and completely when H_2O_2 was added to milk heated to 61.1° C., and that the reagent was not utilized for the oxidation of ascorbic acid in the milk heated to 76.6° C., the temperature at which, according to Zilva (8), the inactivation of peroxidase should be rapid and complete. The reaction was induced again, however, by the addition of horseradish peroxidase solution to non-reactive milk.

These results definitely indicate that peroxidase in milk plays an important

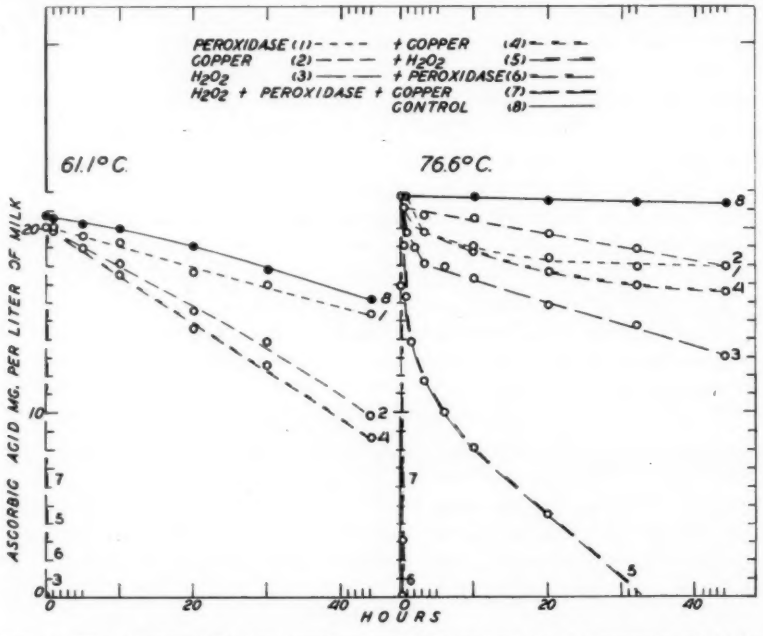

FIG. 1. The effects of the heat treatment of milk and of the subsequently added horseradish peroxidase and copper upon the rate of ascorbic acid oxidation by added hydrogen peroxide.

part in the reaction involving ascorbic acid oxidation in the presence of added H_2O_2. It does not imply, however, that the peroxidase in milk utilizes H_2O_2 directly for the oxidation of ascorbic acid. It is possible that the foregoing reaction proceeds in the milk via a series of reactions, resembling the system which was described by Tauber in his study of the interaction of ascorbic acid and peroxidase (7).

It also could be seen that only a part of the ascorbic acid in the milk heated to 76.6° C., as indicated by the slope of the curve, was oxidized rapidly by H_2O_2 with copper as a catalyst. It has been explained recently that copper acts as

an accelerator of ascorbic acid oxidation to dehydroascorbic acid in milk by atmospheric oxygen and brings the system more quickly to the critical point at which the reaction is forced to deviate from its original course. This critical point was thought to be governed primarily by the ascorbic-dehydroascorbic acids equilibrium (5). This statement was supported by the observations showing that the reaction responsible for the breakdown of the lipid component of the milk system, reaction which manifests itself by a rapid development of the intense tallowy flavor, is initiated more readily when a certain pressure between these two forms of vitamin C has been established. Since, in the course of ascorbic acid oxidation by H_2O_2 with copper as a catalyst, dehydroascorbic acid, as determined by the Gunsalus and Hand method (2), accumulates in the system, it would be logical to assume that the establishment of an ascorbic-dehydroascorbic acid pressure caused the reaction to slow down. As before, it resulted in the promotion of the reaction which produces the off flavors in milk. This secondary reaction was not promoted when ascorbic acid was oxidized with hydrogen-peroxide-peroxidase, either natural or added.

In addition, it should be pointed out that when H_2O_2 was added to milk in excess of the amount needed to oxidize its ascorbic acid content (from 0.03 to 0.12 ml. of 30 per cent H_2O_2 per liter of milk), the subsequent heating for 30 minutes at 61.1° C. invariably resulted either in retardation or inhibition of ascorbic acid oxidation by H_2O_2, as determined by the addition of these reagents to milk after the heat treatment.

CONCLUSIONS

Evidence is presented to show that peroxidase in milk might be responsible for the quick conversion of ascorbic acid to dehydroascorbic acid by added hydrogen peroxide. The data show that the reagent was not utilized for the oxidation of ascorbic acid in the milk heated to 76.6° C., and that the reaction could be induced again by the addition of plant peroxidase.

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PRESERVATION OF DAIRY PRODUCTS FOR THE PHOSPHATASE TEST

GEORGE P. SANDERS AND OSCAR S. SAGER

Division of Dairy Products Research Laboratories, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture, Washington 25, D. C.

Results of recent investigations have shown that the alkaline phosphatase of cow's milk is very stable in dairy products that have not undergone spoilage. For example, it was shown (7) that the enzyme retains a large proportion of its activity, sufficient for determination as an index of pasteurization, in Cheddar cheese which had been held in storage for several years. High activity likewise has been found, after storage for many months under usual refrigeration conditions, in samples of soft and semi-soft cheeses, butter, ice cream and sherbet made from under-pasteurized milk or cream. Scharer (10) showed that the enzyme in milk retains nearly all of its activity under storage conditions for considerable periods, in some instances as long as 7 to more than 10 days. Results of research in the authors' laboratories have demonstrated also that fluid dairy products and perishable soft cheeses, prepared for consumption while fresh, retain their phosphatase activity for a much longer time if stored under refrigeration conditions adequate to effectively retard spoilage than if kept at room temperature.

However, when spoilage takes place, considerable loss of phosphatase activity occurs. Scharer (10) pointed out that this loss may be due to the increase in acidity coupled with the inadequacy of the buffer under such extreme conditions to maintain the optimum pH for activity of the enzyme. Scharer also pointed out that it is difficult to obtain a representative sample of a milk that has spoiled. Results here have verified the hypothesis that excessive acidity causes partial inactivation of phosphatase. Furthermore, the results have demonstrated also that the increase in acidity makes it necessary, for reliable results, to modify the test by increasing the concentration and the alkalinity of the buffer in order to produce a pH of approximately 10, which is optimum for enzymic hydrolysis (8) (9).

In addition to the partial inactivation of the enzyme that results from a marked decrease in pH, our results have shown that additional inactivation occurs in instances in which souring and spoilage are accompanied by proteolysis. This inactivation suggests a decomposition of the base protein of the phosphatase by proteolytic enzymes produced by microorganisms. Like the inactivation produced by heat, this loss of activity accompanying proteolysis cannot be restored.

Conversely, in some instances sufficient microbial phosphatase may be produced by abundant growth of certain microorganisms during spoilage of dairy products to yield so-called "false positive" tests, thus seriously complicating the results and the interpretation thereof.

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Hahn and Tracy (2) investigated the use of mercuric chloride, sodium salicylate, sodium tetraborate (borax), hydrogen peroxide and formaldehyde for preserving fluid milk to be tested for phosphatase. They kept samples at room temperature for 3 days and used the Scharer laboratory method for testing. They concluded that mercuric chloride was the only one of these chemicals which was satisfactory. The concentration of mercuric chloride used was stated to be one tablet in one-half pint of milk. Newlander (6) described two sizes of tablets, one containing 0.225 g. and another 0.45 g. of the chloride. Thus, the concentration of mercuric chloride used in milk by Hahn and Tracy may have been either 0.09 or 0.18 per cent.

Neave (5) found that mercuric chloride in a concentration of 0.01 per cent "increased the keeping quality of the milk from 1 to 8 days at 68° F." and had no significant effect on milk phosphatase in samples tested by a modification of the Kay-Graham method. However, a concentration of 0.02 per cent, which apparently is much less than that used by Hahn and Tracy, had some destructive effect on the enzyme. Later, Scharer (11) reported that one tablet of mercuric chloride in one-half pint of milk completely destroyed the enzymic activity in 1 day in pasteurized milk with 0.5 per cent of raw milk added. His results indicated that 2 g. of borax per one-half pint of milk, a concentration of approximately 0.8 per cent, decreased the phosphatase activity in the test from 5 to 3.5+ units in 3 to 5 days, but that it was the only effective common preservative.

Barber and Frazier (1) stated that mercuric chloride does not interfere with the test. They used 3 per cent of it to inhibit bacterial growth in pasteurized cream containing phosphatase-producing bacteria, and the results of phosphatase tests after storage were negative.

Mucciolo and Cerveira (4) reported that a concentration of 0.005 per cent of formaldehyde had no appreciable effect on the enzyme in milk stored at room temperature for 72 hours. However, the accuracy of their results is not conclusive, since the phenol values stated to be unaffected by the preservative were reported as "more than 5 Scharer units," a value much too low to indicate quantitatively the actual phosphatase activity of raw milk.

The use of chloroform was recommended by Julien *et al.* (3) as a result of a study of various chemicals for the preservation of samples of cheese to be tested for extraneous matter.

Because quantitative methods for precisely measuring phosphatase activity in dairy products were not available, it was not possible until recently to secure accurate information concerning the kinds, concentrations and inhibitory effects of the chemicals that might be used to preserve dairy products for the test. The quantitative phosphatase test developed in these laboratories (8) requires the use of some reagents that are different from those used in earlier tests and involves different and more precise control of the pH, as was pointed out earlier (9). In view of these modifications, it was conceivable that the newer test might give different and more conclusive results.

In view of the need for increasing the usefulness and the application of the test for all dairy products as an index of pasteurization, and because of the importance of preserving samples that require shipment without refrigeration to a laboratory for testing, it was desirable to investigate the use of preservatives with the Sanders-Sager test (8). The objectives of this research were to determine the kinds and concentrations of various chemicals that would preserve milk and some other milk products for various periods of time at room temperature; the possible inhibitory effects of various preservatives on the activity of the enzyme; and the possible interfering effects of preservatives in the test.

METHODS

The milks used were fresh and of high quality and were taken from the composite milks obtained from a large herd. All samples were prepared by pasteurizing a large part of each mixed lot and then adding raw milk in a proportion of 2.5 per cent. This was done principally to reduce the enzymic activity to a range in which the results of tests could be determined without making dilutions. Thus, the enzymic activities as well as the effects of preservatives were comparable with those that would be found in grossly under-pasteurized milk and in pasteurized milk contaminated with considerable raw milk.

Seven lots of milk were used, and 15 to 18 samples, with various preservatives in different quantities, were prepared from each lot. Thus, more than 110 samples were tested during storage. The flasks and stoppers were sterilized, 100 ml. of milk put in each flask, the preservative added and the contents mixed. The pH values were determined and phosphatase tests were begun within an hour after the addition of preservative. Subsequent pH and phosphatase determinations were made after storage at room temperature, which was between 25 and 29° C., for 1, 3, 5, 7, 10, 14 and 21 days, or until extreme spoilage occurred. A sample of milk from each lot was stored at 3 to 5° C. as an untreated, refrigerated control and was tested at similar intervals. The samples were examined daily for physical evidences of spoilage, including lumping of the fat, separation of whey, formation of gas, curdling and unclean odors.

In some instances in which the phosphatase values increased during storage, *i.e.* in a few samples containing borate, tests for microbial phosphatase were conducted by heating 1 ml. of the product in a tube for 5 minutes at 70° C. to destroy the milk enzyme, cooling to room temperature and then conducting the test in the usual way. A positive test on a sample pasteurized thus is a reliable indication of the presence of microbial phosphatase, *i.e.* of a so-called false positive result. Microbial-phosphatase controls were prepared by adding 1 ml. of the appropriate barium borate-hydroxide buffer (pH 10.6) to 1 ml. of milk and heating the mixture (pH approximately 9.5) in boiling water for 5 minutes to destroy microbial phosphatase, cooling and conducting the test.

The samples of cheese were plugs of Cheddar and Swiss cheese, weighing approximately 10 g., taken with a trier. They were placed in 1- by 8-inch test tubes, containing plugs of cotton moistened with chloroform, as described by

Julien *et al.* (3). Various quantities of chloroform, from 0.5 to 3 per cent of the volume of the containers, were used. The tubes with samples were stoppered tightly and kept at room temperature. Similar samples, without preservative, were stored at 3 to 5° C. as controls.

Determinations of the pH values of fluid samples were made with a Beckman glass-electrode pH meter. Phosphatase tests were made by the Sanders-Sager method (8).

RESULTS

The preservatives that were tested experimentally in milk at room temperature are listed below, with the respective concentrations used and the numbers of days that elapsed before spoilage occurred, as indicated by physical

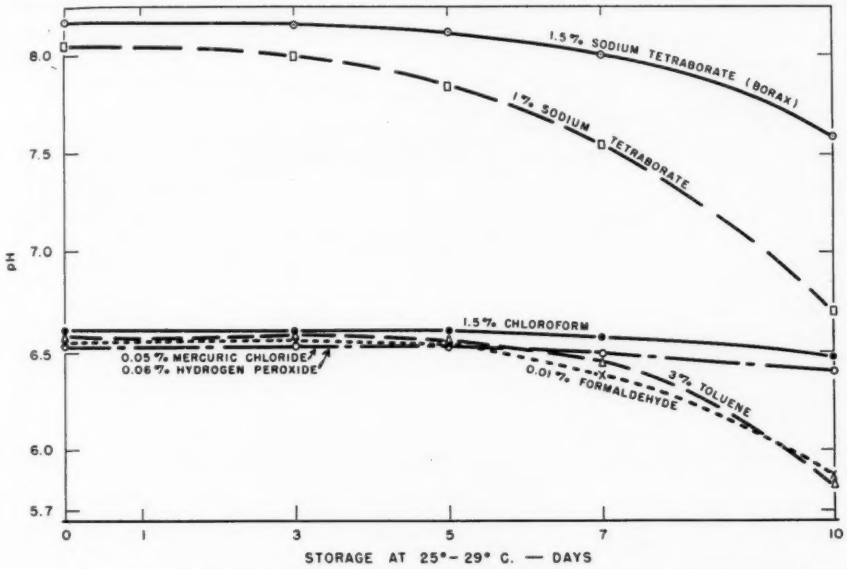

FIG. 1. Minimal concentrations of preservatives required to preserve milk for 7 to 10 days (2.5 per cent of raw milk in pasteurized milk).

changes, principally curdling, and by a relatively steep slope of the pH curve to approximately 5 or slightly lower.

Chloroform: 0.25 per cent, 2 days; 0.5 per cent, 2–3 days; 1 per cent, 6–10 days; 1.5 per cent, 10–21 days; 2 per cent, > 21 days.

Toluene: 1 per cent, 2 days; 2 per cent, 6 days; 3 per cent, 8–12 days; 3.5 per cent, > 21 days.

Sodium tetraborate (borax): 0.25 per cent, 3–4 days; 0.5 per cent, 4–7 days; 0.75 per cent, 8–10 days; 1 per cent, 8–14 days; 1.5 per cent, 10–18 days; 2 per cent, 14– > 21 days.

Sodium metaborate: 0.25 per cent, 4 days; 0.5 per cent, 7 days; 0.75 per cent, 11 days; 1 per cent, 13 days.

Sodium tetraborate-sodium metaborate mixture, equal parts by weight: 0.25 per cent, 3–4 days; 0.5 per cent, 4–6 days; 0.75 per cent, 7–10 days; 1 per cent, 8 days; 1.5 per cent, 10 days; 2 per cent, 13 days.

Formaldehyde: 0.005 per cent (*i.e.*, 0.0125 per cent of a 40 per cent solution by volume), 4–7 days; 0.01 per cent, 6–14 days; 0.03 per cent, 14–21 days; 0.05 per cent, 16 days; 0.1 per cent, $>$ 21 days.

Mercuric chloride: 0.005 per cent, 2 days; 0.01 per cent, 3–6 days; 0.025 per cent, 6–7 days; 0.05 per cent, 7–14 days; 0.1 per cent, $>$ 21 days.

Hydrogen peroxide: 0.015 per cent (*i.e.*, 0.05 per cent of a 30 per cent solution), 2 days; 0.03 per cent, 3–4 days; 0.06 per cent, 8–16 days; 0.09 per cent, 10–21 days; 0.15 per cent, $>$ 21 days.

Correlation of the pH data with the results of physical examinations of the samples showed that changes in pH generally were a reliable criterion of the

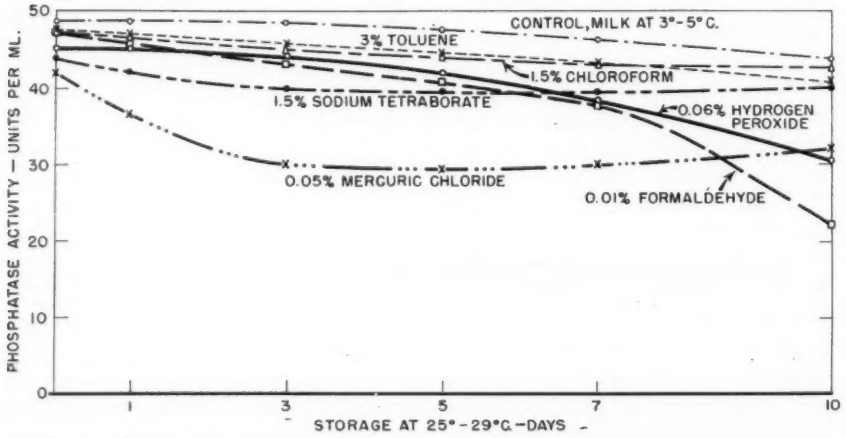

FIG. 2. Effects of preservatives on phosphatase activity of milk in storage for 10 days (2.5 per cent of raw milk in pasteurized milk).

physical changes indicative of spoilage. The effectiveness of the chemicals for preserving milk was evaluated, therefore, on the basis of changes in pH. Illustrative data showing the minimal concentration of each chemical that was effective in preserving the milk adequately for testing, without any marked, undesirable changes in physical characteristics, for periods of 7 to 10 days are presented in figure 1, and for periods of 14 to 21 days in figure 3.

The results of phosphatase tests on these samples, showing the inhibitory effects on the phosphatase activity produced by the concentrations of chemicals indicated in figures 1 and 3, are presented in figures 2 and 4, respectively.

The phosphatase values of the controls stored at 3 to 5° C. without preservative, also shown in figures 2 and 4, decreased in 10 days from an average of 48.5 to 43.5 units per ml., a loss of 10 per cent; and during the same period the pH values decreased from an average of 6.60 to 6.30. During 21 days the

average loss in enzymic activity in the controls was 20 per cent, and the pH decreased to an average of 5.20. These controls generally curdled during the third week.

All of the chemicals tested, when used in suitable concentrations, were effective in preserving the samples of milk for the periods of time indicated. However, they differed greatly in the extent to which they inhibited the enzyme. All of the chemicals tested, except chloroform and toluene, produced a considerable decrease in phosphatase activity. The increasing order of their destructive effects was as follows: chloroform, toluene, sodium tetraborate (borax), formaldehyde, mercuric chloride and hydrogen peroxide.

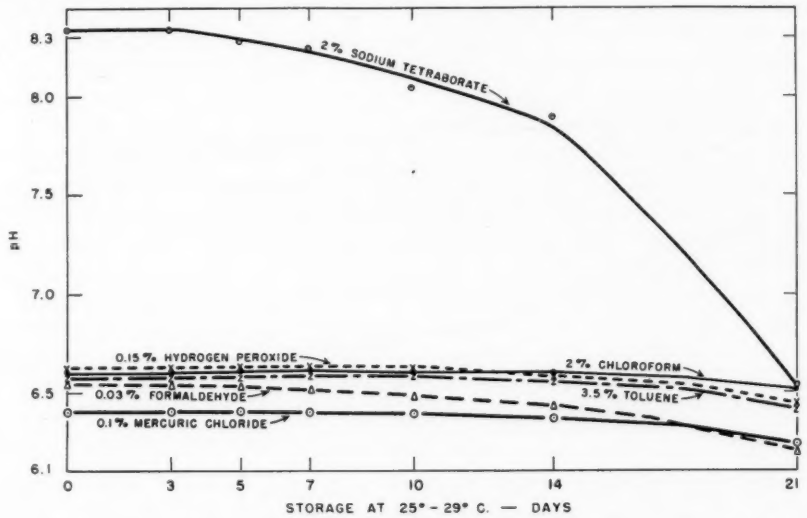

FIG. 3. Minimal concentrations of preservatives required to preserve milk for 14 to 21 days (2.5 per cent of raw milk in pasteurized milk).

Only chloroform and toluene had no seriously inhibitory effects on the enzymic activity. The use of 1.5 to 2 per cent of chloroform or 3 to 3.5 per cent of toluene at 25 to 29° C. generally produced no more or very little more inactivation than was incurred under refrigeration at 3 to 5° C. Chloroform is considered to be more suitable than toluene as a preservative, because it is effective in lower concentration and also because it effectively prevents lumping of the fat for an indefinite period, especially when used in a concentration of 1.5 per cent or greater. Moreover, both have distinctive odors, thus reducing greatly the danger of accidental poisoning.

Sodium tetraborate, in concentrations sufficient to preserve milk, not only inhibited the enzyme more than did either chloroform or toluene (fig. 2 and 4), but also interfered in the test because of its marked buffering effect. When it was present, the pH values in the test generally were about 9.7 instead of within the optimal range of 9.85 to 10.2 (8, 9). Thus, it was necessary to modify the

test by using a more concentrated barium borate buffer of greater alkalinity. Attempts to produce optimal pH conditions in the test by substituting sodium metaborate, which buffers at a higher pH value, were not successful. Moreover, the metaborate was not found to be superior to the tetraborate as a preservative.

Increases in phosphatase values (not included in figures), beginning generally at 10 to 14 days, were found in occasional instances in only the samples containing borates, and tests made of portions of these samples following pasteurization at 70° C. for 5 minutes showed that the increases were due to microbial phosphatase. This condition may have resulted from the activity of phosphatase-producing organisms whose growth may be favored by the relatively high pH produced in milk by borate (figs. 1 and 3).

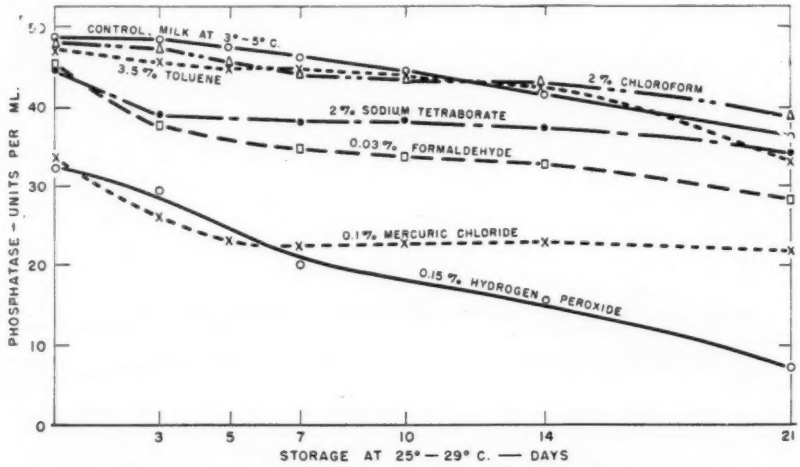

FIG. 4. Effects of preservatives on phosphatase activity of milk in storage for 21 days (2.5 per cent of raw milk in pasteurized milk).

Figures 2 and 4 also show that the enzymic activity was inhibited appreciably by 0.01 to 0.03 per cent of formaldehyde and more markedly by 0.05 to 0.1 per cent of mercuric chloride and also by 0.06 to 0.15 per cent of hydrogen peroxide. The results of the tests begun within an hour after the addition of preservatives show that the inhibitory effects largely are immediate, regardless of storage, and are attributable to chemical effects of the preservatives upon the enzymic activity.

Considerable lumping of the fat prior to curdling occurred with peroxide, mercuric chloride and formaldehyde, and some lumping occurred with tetraborate and toluene, making it relatively difficult to secure homogeneous samples after storage. The use of chloroform eliminated this difficulty.

Samples of hard cheese, stored at room temperature in stoppered tubes containing chloroform, showed no evidence of growth of mold within 3 weeks with

a quantity of chloroform equal to 0.75 per cent or more of the volume of the container, and none within 1 month with 1 per cent or more. The largest decrease in phosphatase activity in 1 month at room temperature in those in which 1 per cent of chloroform had been added was 8 per cent, and the largest decrease in 1 month with 1.5 per cent was 6 per cent. No microbial phosphatase could be detected in tests made on samples taken from the surfaces of the preserved cheese.

Some development of mold was found on the control samples of cheese stored at 3 to 5° C. without chloroform, beginning generally during the late part of the third week on the Cheddar samples and somewhat later on the Swiss samples. No evidence of microbial phosphatase could be detected in or on the surfaces of the refrigerated samples prior to 1 month.

The results with chloroform conformed with those of Julien *et al.* (3) to the effect that it is an ideal preservative.

It was pointed out by Scharer (11) that salicylates are not suitable for preserving samples to be tested for phosphatase, because salicylates react with 2,6-dibromoquinonechloroimine (BQC), producing the blue color of indophenol. This conclusion was verified in our experiments with sodium salicylate. Likewise, cresols, resorcinols and naphthols were found unsuitable, because they also yield interfering colors.

Without exception, samples to which a preservative has been added should be labeled plainly in the following or a similar manner: "*Poison, preservative added.*"

It should be recognized that concentrations of preservatives greater than those described herein may be necessary to preserve entirely raw milk for comparable periods of time under summer conditions.

SUMMARY AND CONCLUSIONS

Research on the preservation of milk for the phosphatase test showed that each of the chemicals listed below will preserve fresh milk (in these experiments, mixtures of 2.5 per cent of fresh, raw milk in fresh, pasteurized milk) for periods as long as 10 days to 3 weeks under summer conditions at room temperature. The preservatives tested, arranged in increasing order of their inhibitory effects on milk phosphatase, were as follows: 1.5 to 2 per cent of chloroform, 3 to 3.5 per cent of toluene, 1.5 to 2 per cent of sodium tetraborate (borax), 0.01 to 0.03 per cent of formaldehyde (0.025 to 0.075 per cent of a 40 per cent solution by volume), 0.05 to 0.1 per cent of mercuric chloride, and 0.06 to 0.15 per cent of hydrogen peroxide (0.2 to 0.5 per cent of a 30 per cent solution).

Chloroform was more effective than toluene as a preservative in the same concentration. Neither inhibited the enzyme appreciably. Other advantages of chloroform are that it prevents the fat emulsion from breaking, thus eliminating the separation of lumps of fat on the surface, and its presence can be detected very easily because of its distinctive odor, thus reducing greatly the danger of accidental poisoning.

Hydrogen peroxide, mercuric chloride and formaldehyde produced serious chemical inhibition of the activity of milk phosphatase. Sodium tetraborate had only a slight inhibitory effect, but the quantity of it necessary for preservation yielded in milk pH values of approximately 9.7 in the phosphatase test instead of optimal pH values of 9.85 to 10.2, and thus it interfered in the test. This excessive buffering interference could not be corrected adequately by substituting the more alkaline sodium metaborate as a preservative.

Phenolic compounds, including salicylates, cresols, resorcinols and naphthols, should not be used as preservatives in connection with the test, because they react with BQC and yield blue or other interfering colors.

It is recommended that 1.5 to 2 per cent of chloroform be used to preserve fluid dairy products for the phosphatase test. For solid samples, including cheese, it is recommended that chloroform, in a quantity equal to 1.5 to 2 per dent of the volume of the container, be put on a wad of cotton in the sample tube or jar with the sample and that the container be stoppered tightly.

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FROZEN HOMOGENIZED MILK VI.
THE USE OF STABILIZERS IN FROZEN HOMOGENIZED MILK

C. J. BABCOCK,[1] CAPTAIN JOSEPH N. STABILE, MSC, USA, ERNEST S. WINDHAM, AND COLONEL RAYMOND RANDALL, VC, USA

Veterinary Division, Army Medical Center, Washington 12, D. C.

According to Tressler and Evers (10), the freezing of whole milk and condensed milk is not being practiced on any large commercial scale, but since there is a growing demand for this product the industry should develop into a sizable one. The use of frozen homogenized milk to supply fresh milk to patients on hospital ships during World War II (1) has caused considerable interest in this product. However, there are two major problems which must be overcome before homogenized milk can be held for long periods of time in the frozen state before use. They are the development of methods for the preservation of flavor quality and the prevention of separation of the milk solids which is evident, at times, on thawing.

While the use of stabilizers in ice cream and evaporated milk is widespread, reports of their use in frozen homogenized milk have not been found. However, Seekles and Smeets (9) report that the stabilization of milk without altering its pH may be affected by adding certain substances which cause the activity of the calcium ions to decrease. These workers found that sodium citrate slightly decreased the rate of flocculation, and that calcium-sequestering polyphosphates definitely retarded it but introduced a readily detectable astringent flavor. The desirability of delaying the appearance of protein flocculation and flavor defects is strongly indicated in reports of previous investigations (1, 2, 3, 4, 5, 6, 8, 11, 12).

EXPERIMENTAL

The present experiments were undertaken to determine the ability of certain chemicals to act as stabilizers and thereby materially increase the storage life of frozen homogenized milk. Homogenized milk with a fat content of 3.8 per cent, pasteurized by holding at 155° F. for 30 minutes and packaged in one-half pint paper containers by a commercial dairy was used. With the exception of ascorbic acid, the quantity of each stabilizer required to impart a detectable flavor to the milk was determined for the first series. A slightly smaller quantity then was added to the milk to be frozen. This amount of hydrogen peroxide used as a stabilizer, however, caused the milk to have an astringent flavor after it had been frozen and thawed and failed to reduce materially the bacterial content of the milk either before or after freezing.

The chemicals used and their concentration per liter of homogenized milk were as follows: (a) ascorbic acid, 0.1 g.; (b) pectin, 1 g.; (c) calcium chloride, 0.5 g.; (d) urea, 3 g.; (e) carboxyl methyl cellulose, 0.5 g.; (f) sodium citrate, 2 g.; (g)

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[1] Formerly Lieutenant Colonel, SnC, AUS. Present address, Dairy Branch, Production Marketing Administration, U. S. Department of Agriculture.

TABLE 1

Influence of various chemicals on the stability of frozen homogenized milk stored at −17.8° C. (0° F.)

Storage time	Chemical added								
	Control	Ascorbic acid	Pectin	Calcium chloride	Urea	Carboxyl methyl cellulose	Sodium citrate	Disodium phosphate	Hydrogen peroxide
	Degree of separation (Ml. sediment/50 ml. milk)								
(*days*)									
5	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03
15	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	.....
27	0.02	0.01	0.01	0.02	0.02	0.01	0.02	0.02	.....
35	0.02	0.01	0.01	0.03	0.02	0.02	0.02	0.02	.....
44	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	.....
55	0.03	0.03	0.10	0.02	0.20	0.70	0.02	0.60	0.02
65	1.50	0.90	0.20	0.60	0.20	3.50	0.05	2.00	.....
75	0.40	0.40	0.70	1.80	0.20	1.10	0.04	3.00	.....
85	0.90	0.90	1.20	2.00	0.20	2.00	0.03	5.00	.....
95	3.00	0.90	1.50	2.80	0.30	4.50	0.04	10.00	.....
105	.....	1.20	5.00	6.00	0.50	10.00	0.03	6.50	0.20
115	.....	1.80	2.20	2.20	0.40	7.50	0.03	11.00	1.50
125	.....	1.60	1.20	6.00	0.50	13.50	0.10	12.00	4.50
135	.....	5.50	6.50	9.00	1.20	11.00	0.20	14.00	.....
145	.....	12.00	13.00	13.00	2.50	14.00	0.90	15.00	.....
155	.....	.....	.....	.....	.....	.....	0.50	.....	.....
175	.....	.....	.....	.....	.....	.....	1.20	.....	.....
	Flavor								
5	Normal	Normal	Normal	Normal	[a]Sl. flat	Sl. abnormal	Sl. flat	Sl. flat	[e]Ab. astringent
15	Normal	Normal	Normal	Sl. salty	Sl. flat	Normal	Normal	Sl. flat	.....
27	Normal	Normal	Normal	Sl. salty	Sl. flat	Normal	Normal	Sl. flat	.....
35	Normal	Normal	Normal	Normal	Sl. flat	Normal	Normal	Normal	.....
44	Normal	Normal	Normal	Normal	Sl. flat	[d]Tr. oxidized	Normal	Normal	.....
55	Normal	Normal	Normal	Normal	Sl. flat	Normal	Normal	Sl. flat	Ab. astringent
65	[b]V. sl. old	Normal	Normal	Normal	Sl. flat	Sl. oxidized	Normal	Sl. old	.....
75	Sl. oxidized	Normal	Sl. oxidized	Sl. oxidized	V. sl. old	Sl. oxidized	Normal	Old	.....
85	V. sl. old	Normal	Oxidized	V. sl. oxidized	V. sl. old	Sl. oxidized	Normal	Old	.....
95	V. sl. old	Normal	Oxidized	Sl. oxidized	[d]Tr. oxidized	Oxidized	Normal	Old	.....
105	.....	Normal	Oxidized	Sl. oxidized	Tr. oxidized	Oxidized	Lacks freshness	Old	Abnormal
115	.....	V. sl. old	Oxidized	Oxidized	Oxidized	St. oxidized	Lacks freshness	Old	Abnormal
125	.....	Sl. old	Oxidized	St. oxidized	Oxidized	St. oxidized	V. sl. old	Old	Abnormal
135	.....	Sl. old	Oxidized	St. oxidized	Oxidized	St. oxidized	Sl. old	Sl. oxidized	Sl. oxidized
145	.....	V. sl. oxidized	[c]St. oxidized	St. oxidized	.....	St. oxidized	Old	Oxidized	.....
155	.....	.....	.....	.....	.....	.....	Old	.....	.....
175	.....	.....	.....	.....	.....	.....	Old-chalky		

[a]Sl. = slightly; [b]V. sl. = very slightly; [c]St. = strongly; [d]Tr. = trace; [e]Ab. = Abnormal

disodium phosphate, 1 g.; and (h) 30 per cent hydrogen peroxide, 1 ml. An additional group of samples to which no chemical was added served as a control group.

The samples were frozen and stored at about –17.8° C.[2]. They were removed and thawed for examination at intervals of about 10 days beginning with the fifth storage day. The degree of separation of the milk was measured by determining the quantity of sediment in 50 ml. by centrifuging, as was done in previously reported experiments (1). Flavor determinations again were made by a panel of three men experienced in milk judging.

Table 1 shows that sodium citrate was of greater value as a stabilizer than any of the other substances used. Separation was not noticeable in the milk samples containing sodium citrate until they had been stored for 145 days, and flavor deterioration was not evident until at least 105 days. Noticeable separation had taken place and flavor deterioration had become evident in the control samples after a storage period of 65 days. The milk with added ascorbic acid showed separation at the same storage age as did the control samples, but its addition preserved the flavor slightly longer than did the addition of sodium citrate. The milk samples containing pectin, calcium chloride, carboxyl methyl cellulose and disodium phosphate each showed separation and flavor deterioration at about the same time as the control group. Urea and hydrogen peroxide each delayed the occurrence of separation but did not help to preserve the flavor of the milk.

To further establish their value as stabilizers in frozen homogenized milk, sodium citrate and sodium citrate in combination with ascorbic acid were used under different storage conditions. The homogenized milk used was of the same quality and was packaged in the same manner as that used in the first series. The three equal groups consisted of a control group, a group to which 2 g. of sodium citrate per liter was added and a group to which, in addition to the sodium citrate, 100 mg. per liter of ascorbic acid was added.

It has been shown that if homogenized milk is frozen and held for short periods of time at a low temperature and then moved and held in a frozen state at a much higher temperature, the stability of the milk is affected adversely (1). To simulate this adverse condition of storage, each of the three main groups was divided into sub-groups, first frozen and then treated as follows: A_1 held at a constant temperature of – 11.5° C.; A_2 held at – 17.5° C.; B_1 held at – 27.5° C. and after 5 days held at – 17.5° C.; B_2 held at – 27.5° C. and after 25 days held at – 17.5° C.; C_1 held at – 27.5° C. and after 5 days held at – 11.5° C.; C_2 held at – 27.5° C. and after 25 days held at – 11.5° C.; D_1 held at – 17.5° C. and after 5 days held at – 11.5° C.; D_2 held at – 17.5° C. and after 25 days held at – 11.5° C. Representative samples of all sub-groups were examined at intervals of about 10 days for flavor and sediment.

Section A of table 2 shows that the addition of sodium citrate with ascorbic acid doubled the time that homogenized milk remained normal in appearance and flavor when frozen and stored either at – 11.5° C. or at about – 17.8° C. when compared with frozen milk without the addition of stabilizers. As was shown in table

[2] The thermostat controlling the temperature of the freezer had sufficient lag to cause a maximum temperature variation of about 8° C.

[a]Sl. = slightly; [b]V. sl. = very slightly; [c]St. = strongly; [d]Tr. = trace; [e]Ab. = Abnormal

TABLE 2

Influence of sodium citrate and sodium citrate with ascorbic acid on the stability of frozen homogenized milk

Total storage time	A—Frozen and held at:					
	(A_1) −11.5° C.			(A_2) −17.5° C.		
	Character of sample					
	Control	Sodium citrate	Sodium Citrate with ascorbic acid	Control	Sodium citrate	Sodium Citrate with ascorbic acid
	Sediment in 50 ml. milk					
(*days*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)
47	0.50	0.04	0.02	0.04	0.02	0.01
55	0.70	0.20	0.15	0.50	0.02	0.02
64	0.90	0.20	0.10	0.60	0.02	0.01
74	0.70	0.30	0.25	1.00	0.02	0.02
84	1.40	0.50	0.40	2.40	0.05	0.03
95	2.50	0.30	0.70	1.60	0.03	0.03
104	6.50	0.90	0.50	.....	0.08	0.05
114	.....	1.40	0.60	.....	0.20	0.15
123	8.50	0.70	1.00	11.00	0.10	0.40
140	11.00	1.00	2.00	11.00	0.20	.....
154	10.50	2.00	1.10	12.00	0.80	0.70
	Flavor					
47	Tr. oxidized[a]	Normal	Normal	Normal	Normal	Normal
55	Sl. oxidized[b]	Normal	Normal	Normal	Normal	Normal
64	Oxidized	Normal	Normal	Normal	Normal	Normal
74	Oxidized	Normal	Normal	Normal	Normal	Normal
84	Sl. oxidized	Sl. flat	Normal	Oxidized	Normal	Normal
95	Flat	Normal	Normal	Oxidized	Sl. flat	Normal
104	Sl. oxidized	Flat	Normal	.....	Sl. flat	Normal
114	Oxidized	Flat	Normal	.....	Sl. oxidized	Normal
123	Oxidized	Sl. oxidized	Sl. oxidized	Sl. oxidized	Sl. oxidized	Normal
140	.....	Sl. oxidized	Sl. oxidized	Oxidized	Oxidized	.....
154	.....	Oxidized	Oxidized	.....	Oxidized	Old, v. sl.[c] oxidized
	B—Frozen and held at −27.5° C.; moved to −17.5° C.					
	(B_1) moved after 5 days			(B_2) moved after 25 days		
	Sediment					
25	0.02	.....	.....	.....	.....	.....
35	0.02	.....	.....	.....	.....	.....
47	0.10	0.01	0.02	0.10	.....	.....
55	0.30	0.02	0.02	0.05	0.02	0.02
64	0.50	0.01	0.01	0.30	0.02	0.02
74	1.00	0.02	0.02	0.50	0.02	0.02
84	.....	0.03	0.05	1.00	0.03	0.07
95	.....	0.04	0.03	3.50	0.04	0.03
104	.....	0.07	.....	.....	0.05	0.03
123	.....	.....	.....	.....	0.02	.....
140	.....	.....	.....	.....	0.02	.....

	Flavor					
25	V. sl. flat	.....	.....	.....	.....	.....
35	Normal	.....	.....	.....	.....	.....
47	V. sl. oxidized	Normal	Normal	Tr. oxidized	.....	.....
55	Sl. oxidized	Normal	Normal	Tr. oxidized	Normal	Normal
64	Sl. oxidized	Normal	Normal	Sl. oxidized	Normal	Normal
74	Sl. oxidized	Normal	Normal	Sl. oxidized	Normal	Normal
84	.....	Sl. flat	Sl. oxidized	Sl. oxidized	Normal	Normal
95	.....	Normal	Normal	Sl. oxidized	Normal	Normal
104	.....	Sl. flat	.....	.....	Flat	Normal
123	.....	.....	.....	.....	.....	.....
140	.....	.....	.....	.....	Sl. oxidized	.....

[a] Tr. = trace; [b] Sl. = slight; [c] V. sl. = very slight.

1, the addition of sodium citrate delays separation or flocculation in frozen homogenized milk stored at a constant temperature. When the milk was frozen and stored at – 11.5° C., noticeable separation had occurred in the control samples that were thawed after 55 days storage. This separation was not noticeable in the samples containing sodium citrate until they had been stored from 95 to 105 days. The contral samples stored at about – 17.8° C., showed separation after storage for 64 days. The samples containing sodium citrate, when stored at this temperature, did not show separation for more than 140 days. At each of the storage temperatures used the addition of sodium citrate also delayed the development of abnormal flavors. The development of these flavors was delayed further by adding ascorbic acid to the samples containing sodium citrate. This finding was more pronounced in the samples stored at about – 17.8° C. than it was in those stored at – 11.5° C.

Sections B, C and D of table 2 show that the addition of sodium citrate and ascorbic acid prolonged the time that homogenized milk remained normal in appearance and flavor when it was frozen and held at one temperature and then stored at a higher temperature. When the homogenized milk was frozen and stored for 5 days at – 27.5° C. and then moved to – 17.8° C., or frozen and stored for 5 days at – 27.5° C. and then moved to – 11.5° C., or frozen and stored for 5 days at – 17.8° C. and then moved to – 11.5° C., noticeable separation was delayed from 64 to more than 104 days, from less than 35 to 95 days, and from less than 35 to 84 days, respectively. The appearance of flavor deterioration also was delayed from 47 to 84 days, from 47 to 95 days and from 35 to more than 84 days, respectively.

When the homogenized milk was frozen and stored for 25 days at – 27.5° C. and then moved to – 17.8° C., or frozen and stored for 25 days at – 27.5° C. and then moved to – 11.5° C. or frozen and stored for 25 days at – 17.8° C. and then moved to – 11.5° C., sodium citrate plus ascorbic acid prolonged the period before noticeable separation occurred from less than 47 to more than 140 days, from 47

TABLE 3

Influence of sodium citrate and sodium citrate with ascorbic acid on the stability of frozen homogenized milk (Cont'd)

Total storage time	Character of sample					
	C—Frozen and held at –27.5° C.; moved to –11.5° C.					
	(C_1) moved after 5 days			(C_2) moved after 25 days		
	Control	Sodium citrate	Sodium Citrate with ascorbic acid	Control	Sodium citrate	Sodium Citrate with ascorbic acid
	Sediment					
(*days*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)	(*ml.*)
25	0.10	.....	.....	.....	.....	.....
35	1.00	.....	.....	.....	.....	.....
47	1.90	0.04	0.03	0.50	.....	.....
55	.....	.....	0.20	2.00	0.10	0.10
64	11.00	0.02	0.10	2.70	0.08	0.10
74	10.80	0.15	0.20	2.00	0.10	0.50
84	10.00	0.50	0.20	9.00	0.40	0.40
95	.....	0.40	1.00	.....	0.50	0.35
104	.....	1.00	0.80	.....	0.80	0.45
114	.....	.....	1.20	.....	.....	.....
123	.....	.....	2.70	.....	.....	0.80
140	.....	2.40	1.20	.....	1.90	2.00
	Flavor					
25	Sl. flat	.....	.....	.....	.....	.....
35	Sl. flat	.....	.....	.....	.....	.....
47	Sl. oxidized	Normal	Normal	Sl. oxidized	.....	.....
55	.....	.....	Normal	Oxidized	Normal	Normal
64	Sl. oxidized	Normal	Normal	Oxidized	Normal	Normal
74	Sl. oxidized	Normal	Normal	Oxidized	Normal	Normal
84	Sl. oxidized	Sl. flat	Normal	Oxidized	Normal	Normal
95	.....	Sl. flat	Sl. flat	.....	Sl. flat	Normal
104	.....	Flat	Flat	.....	Normal	Normal
114	.....	.....	Sl. flat	.....	.....	.....
123	.....	.....	Flat	.....	.....	Normal
140	.....	Sl. oxidized	Flat	.....	Sl. oxidized	Oxidized
	D—Frozen and held at –17.5° C.; moved to –11.5° C.					
	(D_1) moved after 5 days			(D_2) moved after 25 days		
	Sediment					
25	0.10	.....	.....	.....	.....	.....
35	0.70	.....	.....	.....	.....	.....
47	1.00	.....	.....	1.0	.....	.....
55	3.00	0.20	.....	2.5	.....	0.30
64	3.50	0.30	0.30	3.5	0.02	0.40
74	3.00	0.25	0.20	5.0	0.50	0.50
84	.....	0.70	0.80	7.0	1.90	1.60
95	.....	1.00	.....	9.0	1.60	1.80
104	.....	1.30	1.60	.....	2.50	2.60
114	.....	.....	.....	.....	.....	.....
123	.....	.....	.....	.....	4.5	.....
140	.....	.....	.....	.....	4.8	.....

	Flavor					
25	Sl. flat	.....	.....	.....	.....	.....
35	Sl. oxidized	.....	.....	.....	.....	.....
47	Sl. oxidized	.....	.....	Tr. oxidized	.....	.....
55	Oxidized	Normal	.....	Oxidized	.....	Normal
64	Oxidized	Normal	Normal	Oxidized	Normal	Normal
74	Oxidized	Normal	Normal	Oxidized	Normal	Normal
84	.....	Sl. flat	Normal	Sl. oxidized	Sl. flat	Normal
95	.....	Sl. flat	.....	Sl. oxidized	Sl. flat	Tr. oxidized
104	.....	Flat	Flat	.....	Flat	Flat
114	.....	.....	.....	.....	.....	.....
123	.....	.....	.....	.....	Sl. oxidized	.....
140	.....	.....	.....	.....	Sl. oxidized	.....

to 74 days and from less than 47 to 74 days, respectively. The appearance of flavor deterioration also was delayed from less than 47 to more than 104 days, from less than 47 to more than 123 days and from less than 47 to 84 days, respectively. The samples of frozen homogenized milk which contained ascorbic acid in addition to sodium citrate remained normal in flavor slightly longer than the samples containing only sodium citrate.

CONCLUSIONS

Sodium citrate in a concentration of 2 g. per l. was found to be of value as a stabilizer in homogenized milk frozen and stored at −17.8° C. Separation was not noticeable in the thawed milk samples containing sodium citrate until they had been stored for 145 days, and flavor deterioration was not evident until they had been stored for at least 105 days. Noticeable separation had taken place and flavor deterioration had begun in the control samples after a storage period of 65 days.

Homogenized milk containing added ascorbic acid in a concentration of 0.1 g. per l., frozen and stored at −17.8° C., showed separation when thawed at the same storage age as the control samples, although its addition preserved the flavor slightly longer than did the addition of sodium citrate alone.

Hydrogen peroxide (30 per cent) in a concentration of 1 ml. per l. and urea in a concentration of 3 g. per l. each delayed slightly the occurrence of separation in homogenized milk frozen and stored at −17.8° C. but did not retard the development of abnormal flavors.

Pectin and disodium phosphate, each in a concentration of 1 g. per l., and calcium chloride and carboxyl methyl cellulose, each in a concentration of 0.5 g. per l., did not delay separation or retard the development of abnormal flavors in homogenized milk frozen and stored at −17.8° C.

The addition of sodium citrate with ascorbic acid to homogenized milk, frozen and stored at −11.5° C. and at −17.8° C. doubled the time the milk remained normal in appearance and flavor when thawed in comparison with the control

samples. The addition of sodium citrate with ascorbic acid also prolonged the time that homogenized milk could be frozen and held at one temperature and subsequently stored at a higher temperature and still be normal in appearance and flavor when thawed.

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THE EFFECT OF A COMBINATION OF PENICILLIN AND STREPTOMYCIN UPON THE LIVABILITY AND BACTERIAL CONTENT OF BOVINE SEMEN[1]

J. O. ALMQUIST,[2] P. J. GLANTZ,[3] AND H. E. SHAFFER[2, 4]
Pennsylvania Agricultural Experiment Station, State College

Earlier investigations at this Experiment Station (1, 2) demonstrated that either penicillin or streptomycin will inhibit the growth of bacteria in diluted bull semen. However, concentrations as high as 2,000 units per ml. of diluted semen of either one alone failed to provide complete inhibition. In both studies organisms resistant to the antibiotic employed were encountered in certain of the diluted semen samples.

It generally is recognized that streptomycin is more effective than penicillin against gram-negative microorganisms and that most strains of gram-positive organisms are more sensitive to penicillin than to streptomycin. Since both gram-positive and gram-negative bacteria are found among the predominating types in freshly collected bovine semen (5, 6, 7), it is possible that penicillin and streptomycin in combination would be more effective than either one alone in controlling bacterial growth in diluted semen.

Thompson (8) and Boeing and Laffer (4) found that penicillin plus streptomycin satisfactorily inhibited bacterial growth when added to a selective medium used for the isolation of fungi. Williams and Plastridge (9) reported that the use of 100 units each of penicillin and streptomycin per ml. of medium controlled all bacterial contaminants sufficiently to permit growth of *Trichomonas foetus*. When used alone, a concentration of 1,000 units of streptomycin per ml. was required to free *T. foetus* from *Pseudomonas aeruginosa*. Literature recently reviewed by Beaudette (3) shows that a combination of penicillin and streptomycin was superior to either one alone for controlling bacterial contamination of various exudates and excretions.

The present paper deals with the effect of a combination of penicillin and streptomycin upon spermatozoan livability and the control of bacterial growth in diluted bovine semen.

EXPERIMENTAL

Effect of penicillin-streptomycin upon spermatozoan livability. Based on the livability studies conducted by Almquist *et al.* (1, 2), the following levels

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[2] Dept. of Dairy Husbandry.

[3] Animal Pathology Laboratory, Dept. of Animal Husbandry.

[4] The authors wish to express their appreciation to Dr. W. T. S. Thorp, now with the National Institute of Health, Bethesda, Md., for his suggestions concerning the conduct of the study.

of penicillin (P) and streptomycin (S) with appropriate controls were selected for testing: 100, 250, 500, 750 and 1,000 units of each antibiotic, 250 units of P plus 750 units of S, 750 units of P plus 250 units of S and 500 units of P plus 1,000 units of S per ml. of diluted semen. The preparations used were the sodium salt of penicillin and streptomycin sulphate.

The semen was collected and handled as previously described (1, 2), using a dilution rate of one part of fresh semen to 24 parts of egg yolk-citrate diluter. The dry preparations of penicillin and streptomycin were dissolved in sterile sodium citrate solution and mixed with egg yolk to provide a diluter with 1:1 ratio of yolk to buffer. The diluted semen was stored at 4.5° C., and motility estimations were made every 2 days for a storage period of 20 days. Bacterial plate counts were made on subsamples taken prior to storage and after storage for 8 days.

TABLE 1

The effect of a combination of penicillin and streptomycin upon the livability of bull spermatozoa (Mean of 13 determinations)

Units per ml. of diluted semen		Per cent motile spermatozoa					
Penicillin	Strepto-mycin	Before storage	After storage at 4.5° C. for				
			4 days	8 days	12 days	16 days	20 days
0	0	65	58	43	33	20	8
100	100	65	58	46	34	22	8
250	250	65	59	46	35	22	10
500	500	65	58	44	35	21	9
750	750	65	57	44	35	18	8
1000	1000	65	57	46	33	18	6
250	750	65	57	45	33	20	8
750	250	65	56	46	34	20	8
500	1000	65	59	47	33	20	8

The 13 ejaculates studied had a mean concentration of 1,022,000 spermatozoa per cubic millimeter, a mean initial motility of 65 per cent active spermatozoa, and a mean methylene blue reduction time of 10.6 minutes.

Table 1 gives the mean motility data for the 13 ejaculates. Analysis of variance involving 1170 motility estimations showed no significant difference between treatments. A small but highly significant ejaculate × treatment interaction was obtained indicating that the various ejaculates responded somewhat differently to the several levels of the antibiotics in their livability during storage.

Procedure used for bacterial studies. Plate counts were determined on undiluted semen, yolk-citrate diluter and diluted semen before storage and after storage for 8 days at 4.5° C. Preparations of materials were timed so that plating procedures could begin as soon as the subsamples were taken. Plating of the fresh material was initiated within 1 hour following treatment with penicillin-streptomycin.

Veal infusion agar containing 4 per cent defibrinated bovine blood was used for determining the approximate numbers of living bacteria. The samples were

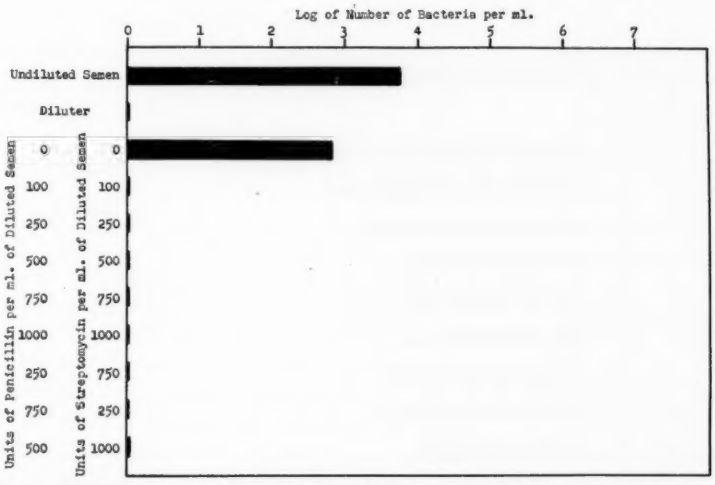

FIG. 1. The effect of a combination of penicillin and streptomycin upon bacterial populations in freshly diluted semen.

plated in dilutions of 1:10, 1:100, 1:1,000 and 1:10,000 and incubated for 48 hours at 37° C. Desoxycholate agar plates incubated for 24 hours at 37° C. were used for determining the number of bacteria belonging to the coliform group. Control plates for sterility tests of the agars and the water used for dilutions also were made.

Effect of penicillin-streptomycin upon bacterial growth in diluted semen. The effect of penicillin-streptomycin mixtures upon bacterial growth in 5 samples

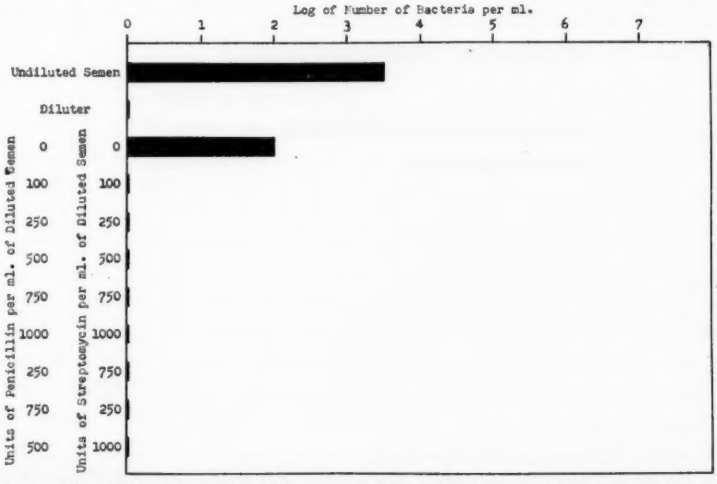

FIG. 2. The effect of a combination of penicillin and streptomycin upon bacterial growth in diluted semen stored for 8 days at 4.5° C.

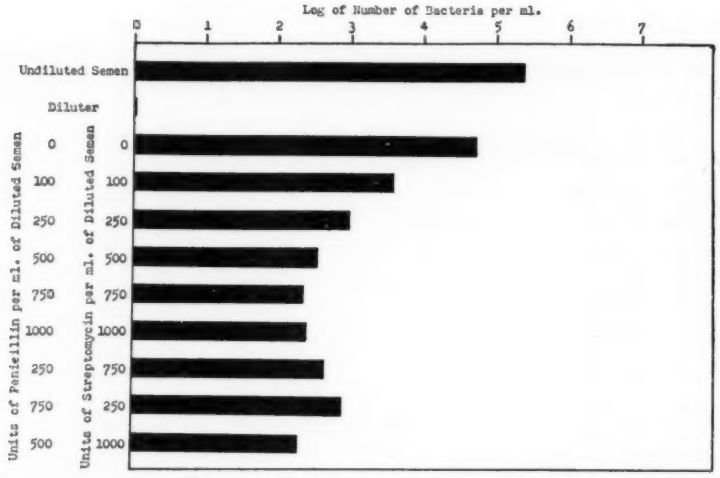
Log of Number of Bacteria per ml.
0 1 2 3 4 5 6 7
Undiluted Semen
Diluter
Units of Penicillin per ml. of Diluted Semen
0 100 250 500 750 1000 250 750 500
Units of Streptomycin per ml. of Diluted Semen
0 100 250 500 750 1000 750 250 1000

FIG. 3. The effect of a combination of penicillin and streptomycin upon bacterial populations in freshly diluted semen inoculated with bacterial cultures.

of diluted semen is shown in figures 1 and 2. It should be noted that logarithmic rather than arithmetic means have been used to present the mean number of bacteria in the 5 semen samples. Since 1:10 was the lowest serial dilution used and at least 25 colonies were required at this dilution before a count was considered significant, any counts below log number 2.40 indicate only that the material was not sterile.

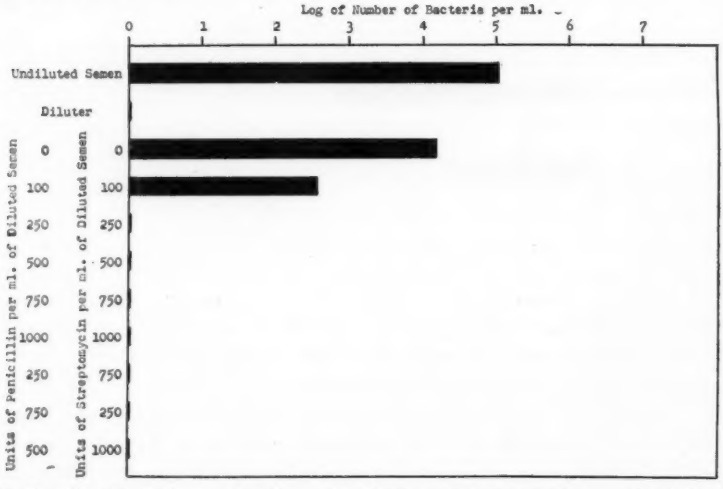
Log of Number of Bacteria per ml.
0 1 2 3 4 5 6 7
Undiluted Semen
Diluter
Units of Penicillin per ml. of Diluted Semen
0 100 250 500 750 1000 250 750 500
Units of Streptomycin per ml. of Diluted Semen
0 100 250 500 750 1000 750 250 1000

FIG. 4. The effect of a combination of penicillin and streptomycin upon bacterial growth in inoculated, diluted semen stored for 8 days at 4.5° C.

As illustrated in figures 1 and 2, treatment of diluted semen with penicillin and streptomycin together effectively controlled bacterial growth at all levels tested in both fresh and stored semen. No growth was observed on plates representing penicillin-streptomycin treated semen. The desoxycholate agar plates also were negative. However, bacterial contamination of the five fresh, undiluted semen samples was very low, as shown by the average plate count of 5,700 per ml.

Effect of pencillin-streptomycin upon bacterial growth in diluted semen inoculated with bacterial cultures. Because of the relatively low plate counts obtained from the first five undiluted semen samples, the next five samples were inoculated with broth cultures of bacteria. Organisms isolated from previous semen platings on veal infusion blood agar were transferred to veal infusion broth. Since no coliform bacteria were isolated from previous samples of semen, strains of *Escherichia coli* isolated from calf feces were used. Broth cultures of five different types of bacteria, including gram-positive rods, gram-positive cocci and gram-negative rods, were utilized for inoculation of the undiluted semen. After incubation for 24 hours at 37° C., 0.1 ml. of each broth culture was removed to 10 ml. of sterile nutrient broth. This dilution was mixed well and 0.5 ml. transferred to 10 ml. of sterile broth. The latter dilution received directly 0.1 ml. of a 24-hour broth culture of coliform bacteria. After mixing thoroughly, 0.2 ml. of the final dilution was removed to the freshly collected undiluted semen.

The effect of a combination of penicillin and streptomycin upon bacterial growth is illustrated in figures 3 and 4. Figure 3 presents the average plate counts of the freshly diluted, inoculated semen. The undiluted semen had a plate count of 270,000 per ml. (log 5.43), while the diluted semen without the antibiotics averaged 52,000 per ml. (log 4.72). There was a decrease to 4,000 per ml. (log 3.60) when 100 units each of penicillin and streptomycin were added to the diluted semen. The number of bacteria per ml. decreased as the concentrations of penicillin and streptomycin increased. It is possible that better bacterial control of the fresh material was not obtained because the serial dilutions tended to dilute the penicillin-streptomycin concentrations below their most effective inhibitory level.

Figure 4 shows the effect of the combined antibiotics upon bacterial growth in inoculated, diluted semen after storage for 8 days at 4.5° C. There were marked decreases in the bacterial plate counts as compared with the same samples plated before storage. At levels above 100 units each of penicillin and streptomycin, negative plate counts consistently were obtained. At the lowest concentration tested, 100 units of each antibiotic, four of the five samples resulted in negative plates, but one sample gave a significant count of 4,000 per ml. The untreated controls had an average plate count of 16,000 per ml. and the undiluted semen had an average count of 120,000 per ml. after 8 days of storage.

Effect of penicillin-streptomycin upon the growth of coliform organisms. As shown in figure 5, levels of penicillin and streptomycin above 100 units per ml. of each gave almost complete inhibition of organisms belonging to the coliform

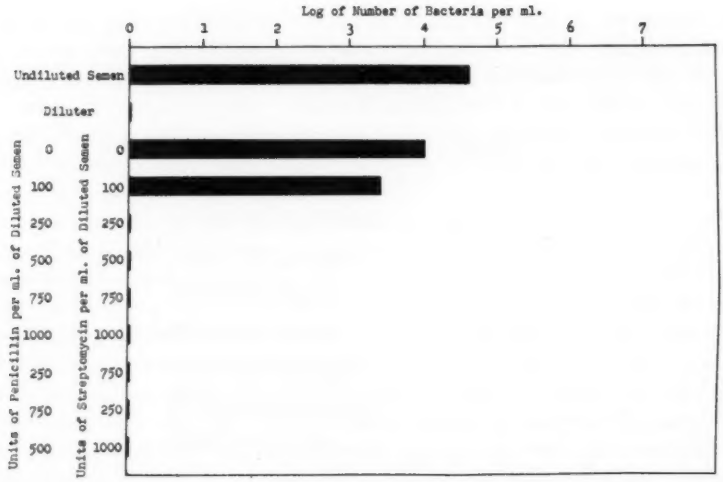

FIG. 5. The effect of a combination of penicillin and streptomycin upon populations of coliform organisms in freshly diluted, inoculated semen.

group. After storage for 8 days, figure 6 shows that all levels of the antibiotics effectively controlled growth of these organisms. When 100 units of each antibiotic per ml. were added, five diluted semen samples showed an average coliform plate count of 2,500 per ml. before storage. However, figure 6 shows that negative counts were obtained at this level when the samples had been stored for 8 days.

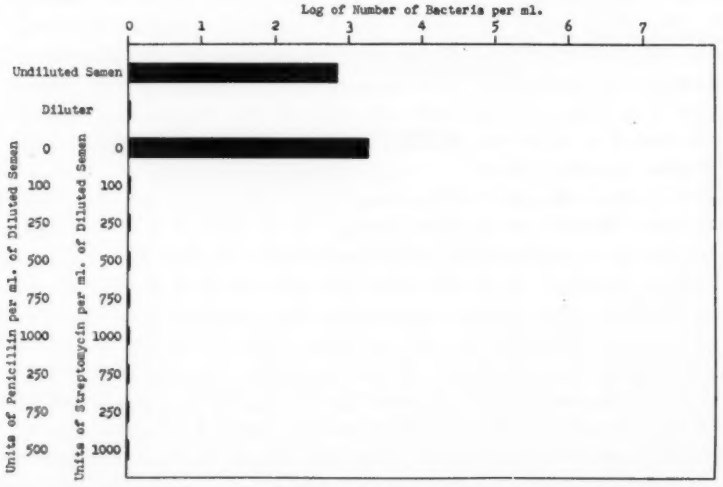

FIG. 6. The effect of a combination of penicillin and streptomycin upon growth of coliform organisms in inoculated, diluted semen after storage for 8 days at 4.5° C.

The coliform plate counts in the undiluted and diluted semen containing no penicillin-streptomycin also decreased during storage. Before storage the inoculated, undiluted semen had a count of 46,000 per ml., while during storage for 8 days there was a marked decrease to 740 per ml. The untreated, inoculated controls decreased from counts of 11,000 per ml. prior to storage to 2,000 per ml. after 8 days of storage.

DISCUSSION

An earlier report by Almquist *et al.* (2) demonstrated that levels of penicillin over 1,000 Oxford units per ml. of diluted semen were injurious to the livability of bull spermatozoa during the time in which practically all semen is used in routine artificial breeding. In similar studies with streptomycin, Almquist *et al.* (1) showed that levels exceeding 1,000 units per ml. of diluted semen significantly reduced spermatozoan livability during a 20-day storage period. In the present study, a combination of penicillin and streptomycin in concentrations as high as 1,000 units of each per ml. of diluted semen did not reduce significantly the ability of the spermatozoa to maintain motility during a storage period of 20 days. Apparently penicillin and streptomycin act independently in their effects upon livability, as the total effect when used together appears to be no greater than when either is used alone.

In comparison with the results of previous studies at this station (1, 2), the use of a combination of penicillin and streptomycin definitely is more effective for controlling bacterial growth in diluted bull semen than either one alone. The results of combined treatment with penicillin and streptomycin showed a marked complementary effect. In spite of inoculation with gram-positive and gram-negative bacteria prior to storage, penicillin-streptomycin treated semen consistently gave negative bacterial counts when plated following storage for 8 days. Field studies now in progress also indicate that negative plate counts may be expected when diluted semen used for artificial breeding is treated with 1,000 units per ml. of both penicillin and streptomycin.

Penicillin and streptomycin assays were not determined in this study. However, Almquist *et al.* (1, 2) found that when used separately these antibiotics were very stable in stored diluted semen. There was no significant loss in streptomycin activity and only a slight decrease in penicillin concentration in diluted semen stored for as long as 16 days. Fertility experiments using penicillin and streptomycin, alone and in combination, are in progress and will be reported as soon as the data are complete.

SUMMARY

1. The use of a combination of penicillin and streptomycin in levels ranging from 100 to 1,000 units of each per ml. of diluted semen did not affect significantly the livability of bull spermatozoa during a 20-day storage period.

2. The use of a combination of penicillin and streptomycin effectively controlled bacterial growth in diluted bull semen.

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ABSTRACTS OF LITERATURE

BOOK REVIEW

78. Milk and dairy products. L. M. LAMPERT. 291 pp. $7.00. Chemical Publishing Co., Inc. 1947.

This book is intended as a summary of the more important points in the chemistry, bacteriology, and the technology of milk and milk products. The illustrations are numerous and add considerably to the value of the presentation. References to the literature permit one to obtain additional information on many of the points covered. The paper and printing are quite satisfactory. The one criticism the reviewer would make is that the brevity of treatment has been carried to the point that the reader might be left without a satisfactory appreciation of the importance of some of the points which are not covered. An example of this is the failure to make any statement about any of the less common varieties of bacteria which may be responsible for some of the more serious defects in the various dairy products. F. E. Nelson

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

79. The early features of bovine mastitis. J. F. MALCOLM and M. M. CAMPBELL. West of Scotland Agr. College, 6 Blythswood Square, Glasgow, C. 2. Proc. Soc. Applied Bact., **1946,** 1: 29–34. 1946.

Observations were made on heifers as they entered the milking herd, examinations being made fortnightly or weekly throughout the first and succeeding lactations. In subclinical cases of mastitis, a rise in the cell content was the first indication of the presence of the disease; milk from mastitis-free animals usually had a cell count of less than 100,000 and frequently less than 50,000. Causative bacteria, e.g. streptococci and staphylococci, frequently were not found in milk having high cell counts, although cows giving such milk showed clinical evidence of mastitis. It was postulated that the pathogenic bacteria often considered as the cause of mastitis may be only secondary invaders which are enabled to obtain a foothold owing to abnormal conditions initially caused by some unknown agent, specific or non-specific in nature. Basis for this also was claimed by the fact that non-specific mastitis in early lactations would in subsequent lactations develop into definite infections. M. L. Speck

80. Sulpha preparation for animal mastitis. D. G. PERKINS. (Assigned to E. R. Squibb and Sons.) U. S. Patent 2,453,259. 3 claims. Nov. 9, 1948. Official Gaz. U. S. Pat. Office, **616,** 2: 439. 1948.

A colloidal suspension of a chemotherapeutic agent of the sulphanilamide type in mineral oil is injected into the animal for treating mastitis. R. Whitaker

81. Some observations on milk fever. A. ROBERTSON, J. W. BURGESS, A. MARR, and BETSY J. C. MILNE. Vet. Record, **60,** 42: 505–508. Oct. 16, 1948.

This study includes data on 25 cases of milk fever. Seventeen of the cases responded normally to calcium therapy, and blood levels for calcium, magnesium and inorganic phosphorus are tabulated for before treatment, soon after treatment and after recovery periods. The average before treatment calcium level was 5.4 mg % as compared to 11.85 mg % in the after recovery period. Magnesium increased slightly from 2.48 to 2.81 mg %, whereas inorganic phosphorus increased from 2.1 to 3.6 mg % in the same periods. In most cases that did not respond to calcium therapy, inflation was used successfully, and the inorganic phosphorus showed a marked rapid rise following inflation. The authors suggested that in some cases of milk fever the treatment effect upon the inorganic phosphorus level may explain the observations of practitioners that at times inflation is a better treatment for milk fever than calcium therapy. R. P. Niedermeier

BUTTER

O. F. HUNZIKER, SECTION EDITOR

82. Water insoluble fatty acids in cream and butter. J. HILLIG and S. W. AHLMANN, Food and Drug Admin., Federal Security Agency, Washington, D. C. J. Assoc. Offic. Agr. Chemists, **31,** 4: 739–749. Nov., 1948.

An investigation was made of certain chemical changes taking place in cream constituents during initial lactic acid souring and in cream in which additional deteriorating changes had occurred. A relationship was found between the occurrence

of unidentified acids (acids other than lactic and volatile acids) in butter and the condition of the cream from which it was churned. The unidentified acids consisted chiefly of water-insoluble acids. When butter was made from decomposed cream, the quantity of water-insoluble acids increased materially. There was no significant change in the content of water-insoluble acids in butter during storage for 5 months at 0° F. The presence of mold tended to increase the water-insoluble acids in butter. Experiments designed to study the partition of water-insoluble acids between butter and buttermilk showed that most of the acids were retained in the butter, very little being found in the buttermilk. Individual cans of cream classified as being decomposed usually contained much larger quantities of water-insoluble acids than cans of cream classified as satisfactory for buttermaking.

F. J. Babel

83. Volatile acids in cream and butter. Part 1. The development of butyric acid during the progressive decomposition of cream. F. Hillig, Food and Drug Admin., Federal Security Agency, Washington, D. C. **Part 2. Butyric acid in commercial creams and butters.** F. Hillig and Dorothy Montgomery, Food and Drug Admin., Federal Security Agency, Washington, D. C. J. Assoc. Offic. Agr. Chemists, **31,** 4: 750–760. Nov., 1948.

Experiments on the decomposition of cream showed that butyric acid frequently was produced in cream when it had reached a point of being classified as unfit for human consumption. The butyric acid was thought to result from the breakdown of lactose and not from the hydrolysis of the fat. When butyric acid was present in cream, a portion usually was carried over into the resulting butter. When the amount of butyric acid present in butter was sufficient to be detected by the method employed (J. Assoc. Offic. Agr. Chemists, **28:** 644. 1945), it was considered that the product had been made from some decomposed cream. An undeterminable amount of butyric acid in butter was considered as an indication that the product had been churned from sound cream. Butyric acid was not found in commercial sweet cream butter.

F. J. Babel

CHEESE

A. C. DAHLBERG, SECTION EDITOR

84. Mikroflora und Struktur des Reifenden Camembertkäses. (The microflora and structure of ripening camembert cheese.) English summary. G. Eigel. Die Milchwissenschaft, **3,** 2: 46–51. 1948.

Camembert cheese was examined during its first 3 weeks of ripening for (a) microbial population, (b) types of flora, (c) pH changes on the surface and within the cheese. Direct microscopic counts were made according to Breed, using Newman stain. Eight smears were prepared from a 10 g. sample and 10 fields counted on each smear. The pH was determined on cheese solutions saturated with quinhydrone and read against a calomel half cell electrode. The distribution of microorganisms in the cheese structure was observed, using a modified procedure of Bondioli which was conducted as follows: small pieces of cheese 0.5 cm. wide were held in a desiccator (containing sulfuric acid) at 4° C. from 1 to 4 days. The firmed cheese was cut 5 μ thick with a microtome, placed on a slide and treated with 1 drop of a mixture of alcohol-xylol-aniline oil. The excess liquid was removed with filter paper, and the dry preparation stained with Newman or Gram stain.

Slight peaks in population were observed at 1–2, 9–11 and 13–15 day old cheese. The first peak was due to acid-forming diplococci and streptococci, whereas the second peak was due to oidium, yeasts and *Penicillium album*, and the third peak was due to an increase in lactobacilli (*Lactobacillus casei*). The pH of the new cheese was the same throughout; the pH increased more rapidly in the outer layer than within, showing a difference of 1.74 units on the 21st day of ripening, namely pH 7.73 as against pH 5.99.

I. Peters

85. Process for making cheese. Z. D. Roundy and H. L. Keil. (Assigned to Armour and Co.) U. S. Patent 2,450,814. 11 claims. Oct. 5, 1948. Official Gaz. U. S. Pat. Office, **615,** 1: 218. 1948.

Cheese, ripened in less than 1 week, is converted into process cheese products by thoroughly incorporating inactive proteolytic pancreatic zymogen material and an activating agent, such as enterokinase, in the milk at setting time. After cutting and firming the curd in the conventional manner, the curd and whey are heated for 10 to 15 min. to 115° (range 110 to 120° F.) to activate the proteolytical enzyme. After the curd is drained and matted, it is cooled and held about 5 days at 35 to 40° F. It then is ready for processing into cheese products. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

86. Milk fat. How the product is manufactured and packaged. Its advantages and potential

uses. T. I. HEDRICK, North Star Dairy, St. Paul, Minn. Milk Plant Monthly, **37,** 10: 80–83. Oct., 1948.

The use of milk fat in the dairy, confection and baking industries has increased tremendously in recent years. This product offers some advantages when compared to butter or cream in that it has a superior keeping quality and is less bulky, thus reducing storage and handling costs.

Different methods of manufacturing milk fat have been perfected, but the method most widely used in the U. S. involves churning of the cream followed by a removal of varying amounts of water and non-fat solids from the melted butter by a filtering, boiling or centrifuging process.

Packaging and storing present the greatest problem, since the fat is susceptible to oxidative and hydrolytic deterioration. The procedures advocated for preventing this decomposition of the fat are: (a) selection of a high quality raw ingredient, (b) avoiding contact of the product or raw materials with metallic catalysts, (c) pasteurization at 170° F. for 20 minutes, which destroys the milk enzyme lipase, (d) maintenance of a moisture content in the finished product under 0.3%, (e) deareation of the finished product to an oxygen content under 1%, (f) storage in nitrogen packed, hermetically sealed cans when the product is to be held above refrigeration temperatures, and (g) the use of antioxidants such as wheat germ oil and N.D.G.A.

J. A. Meiser, Jr.

87. Plastic cream. Its production and uses. R. J. SPIERS, Abbots Dairies, Inc., Philadelphia, Penn. Milk Plant Monthly, **37,** 10: 122–123. Oct., 1948.

To produce a high quality plastic cream, only raw materials of excellent quality can be used, and freedom from copper and iron contamination must be maintained throughout the manufacturing process. Pasteurization after the first separation should take place at 170° F. for not less than 15 min. This is followed by a second separation at 145° F. Packaging should be done only when the product is sufficiently fluid to completely fill the containers. Rapid freezing is essential, thus air blast equipment which circulates large volumes of air at a temperature of 10 to 20° F. below zero is ideal. If these procedures are followed, the product offers no laboratory control problems.

J. A. Meiser, Jr.

88. Manufacture, use and storage of dehydrated sweetened condensed skim milk. A. T. MUSSETT and W. H. MARTIN, Kansas Agr. Expt. Sta., Manhattan. Ice Cream Rev., **32,** 5: 44, 46, 48, 50. Dec., 1948.

A skim milk sugar solution containing around 14 to 22% solids is dried to a powder. The product is said to be more easily dispersed in ice cream mix than powdered milk, is easier to handle and requires less storage space than liquid sweetened condensed milk.

Experimental data pertaining to methods of manufacture indicate that the best powder from the standpoint of color, flavor and texture resulted when the temperature in the drying chamber was maintained at 325° F., with an atomizing pressure of 750 lb. per in.^{2} and when the concentration of the liquid before drying was 14 to 22% solids. When a higher drying temperature (375° F.) was used, a lower moisture content resulted, but the powder prepared in this manner was less desirable from the standpoint of its color and flavor characteristics.

Ice cream prepared with sucrose-sweetened skim milk powder was found to be equal to the control samples with respect to flavor, body and texture and whipping ability. On the other hand, the use of maltose-sweetened powder increased the acidity of the mix, impaired the whipping ability of the mix and resulted in an ice cream characterized by a pronounced malt flavor. Both the maltose and sucrose powders were more easily and completely dispersed in the mix than either non fat dry milk solids or a mixture of pulverized non fat dry milk solids and sucrose.

Storage trials indicate that either glass or tin containers maintained the powders in better condition than when paper containers were used. Samples of the powder were stored for a period of 2 months at either 40° F. or at room temperature in tightly closed glass or tin containers without appreciable change in moisture content, acidity, solubility or flavor characteristics.

W. J. Caulfield

Also see abs. 101, 102, 114.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

89. Pasteurization and its relation to health. JOHN ANDREWS and A W. FUCHS, U. S. Public Health Service, Washington, D. C. J. Am. Med. Assoc., **138,** 2: 128–131. Sept. 11, 1948.

This is an excellent review paper, published at the request of the Council on Foods and Nutrition, Am. Med. Assoc., because of recent published attacks on pasteurization. There is presented a table of milk-borne disease outbreaks

reported during the years 1923–1945, comprised of 955 outbreaks, 40,177 cases, with 804 deaths. Obviously, reporting was not complete, and the data do not include sporadic cases of such diseases as typhoid fever, scarlet fever, septic sore throat, tuberculosis of bovine origin, infantile diarrhea, nor any significant amount of milk-borne brucellosis. In 1945 alone, 5,049 cases of brucellosis and 101 deaths were reported, and about half are believed to have been of raw milk origin. The risk of contracting disease from raw milk is approximately 50 times as great as from pasteurized milk.

Pasteurization is said to be the most important protective measure which can be applied to milk. The process is described briefly. It is stated that more than 75% of the market milk supply of American communities of over 1,000 population is pasteurized. Objections that have been raised to pasteurization are declared to be unsound. Much information is presented to refute claims of loss of nutritive value during pasteurization. The authors conclude that it is obvious that physicians and health authorities are justified in recommending that all milk be pasteurized.

D. P. Glick

90. A new method for fixing, defatting and staining milk and cream films. C. W. Anderson, R. Moehring, and N. O. Gunderson, Dept. of Public Health, Rockford, Ill. J. Milk and Food Technol., **11,** 6: 352–357, 364. Nov.-Dec., 1948.

The authors suggest an improved method over the standard procedure for staining organisms in milk and cream. The slides are defatted and fixed in a chloroform-alcohol mixture for 1 to 2 minutes, dried for 2 minutes and stained with a polychrome methylene blue stain solution from 45 to 60 seconds. Fixing and defatting the milk film are combined in 1 step requiring from 3 to 6 minutes. Granulated particles on the slide are reduced to a minimum, this giving a uniform stained preparation. Decolorization after staining is not necessary since all the bacteria retain the dye. Detailed instructions and preparation of the reagents are given in the paper by the investigators.

H. H. Weiser

91. Some general aspects of the inhibitory streptococci. A. Hirsch, Natl. Inst. Research Dairying, Univ. Reading. Proc. Soc. Applied Bact., **1946,** 1: 26–28. 1946.

The antibiotic activity possessed by strains of *S. lactis* and *S. cremoris* presents certain features which are important both industrially and academically. The presence of such streptococci in starter cultures or their previous activity in milk intended for fermented products can be detected by simple tests. The mode of action of the diplococcin produced by such streptococci apparently is different from that of the sulfonamides and penicillin. The former acts very rapidly without lysis of the cell, indicating a lethal action at the cell surface; the latter act more slowly, indicating interference with anabolic activities within the bacterial cell.

S. lactis diplococcin is active against *S. cremoris,* most other streptococci, other Gram-positive cocci and rods, mycobacteria and certain fungi. *S. cremoris* diplococcin has little or no activity for organisms not belonging to serological group N.

M. L. Speck

92. Viability of Brucella abortus bovis in milk samples from infected cows. G. C. Van Drimmelen, Inst. of Onderstepoort, Pretoria, S. Africa. J. So. African Vet. Med. Assoc., **19,** 2: 58–60. June, 1948.

An intravenous injection of a field strain of *Brucella abortus* was made into a cross-bred Africander X Sussex cow. The cow aborted 1 week after the injection. Milk from this cow was used in the tests. Sediments from 100 cc. samples of milk stored in sterile test bottles on laboratory tables were injected in rats at daily intervals after samples were taken. Rats injected with the culture from milk stored only 1 or 2 d. contracted the disease, whereas the rats receiving the cultures from milk stored for 3 and 4 d. failed to show signs of *Brucella abortus.* The authors point out the futility of taking samples on the open market for *Brucella abortus* examination. It is suggested that milk samples should be taken under hygienic conditions. The samples should be packed in ice if required to ship to a distant laboratory.

K. M. Dunn

93. Fragen der Milchhygiene. (Questions concerned with milk hygiene.) English summary. B. Kemkes. Die Milchwissenschaft, **3,** 4: 108–111. 1948.

The author lists sources of pathogens and shows the manner of their entrance into milk. After 25 years of eradication, 25 to 37% of Germany's milk cows still are infected with tuberculosis and 20% with brucellosis. He points out further the entrance of large numbers of non-pathogenic undesirable microorganisms into milk. Pasteurization is not a remedy for unsanitarily produced milk. Rigid sanitary control and higher milk prices may result in a better quality milk.

The supervision of the milk supply is not a problem of the veterinarians only, but should be the duty of the physicians as well. But because the physicians are overloaded already with various duties, the responsibility of milk super-

vision should be turned over to hygienic bacteriological institutions, such institutions being well qualified for the task. I. Peters

94. The toxicity of certain organic acids to yeast and mold in the presence of fruit juice-syrup mixtures. R. E. Morse, C. R. Fellers and A. S. Levine, Dept. of Food Technol., Univ. of Mass., Amherst. J. Milk and Food Technol., **11,** 6: 346–351. Nov.-Dec., 1948.

The preserving effects of acetic, citric and lactic acids and sodium benzoate were studied when each was combined with fruit juice-syrup mixtures. Acetic acid was more effective against mold than the yeast as compared to the other compounds, while yeast showed the reverse effect. Lactic acid required a concentration of 6.25% to stop yeast growth as compared to 4.58% to prevent mold development. Acetic acid was more toxic to the test organisms than either citric or lactic acids H. H. Weiser

95. Method of fermenting whey. E. R. Engel. U. S. Patent 2,449,064. 10 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, **614,** 2: 415. 1948.

A fermented alcoholic whey product is produced by culturing with yeast and sugar at 58 to 72° F. for 3 d., followed by a series of holding periods at lower temperatures, decantation of the liquid and final aging to produce a liquor of light green color, pleasant taste and bouquet and a milk smoothness. R. Whitaker

96. Chemical engineering unit processes review. Fermentation. H. E. Silcox and S. B. Lee, Merck & Co., Rahway, N. J. Ind. Eng. Chem., **40,** 9: 1602–1608. 1948.

This paper is one of 18 review articles on unit processes. Sections of the fermentation review of interest to the dairy industry include those on riboflavin, penicillin and lactic acid.

B. H. Webb

Also see abs. no. 83, 84.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

97. The cholesterol content of cows' milk. B. Nataf, O. Mickelsen, A. Keys, and W. E. Petersen. Univ. of Minn., Minneapolis and St. Paul. J. Nutrition, **36,** 4: 495–506. Oct., 1948.

The cholesterol content of "winter" and "summer" milks from Holstein, Jersey and Guernsey cows was determined. No ester cholesterol was found. The average cholesterol content of milk from all cows was 11.4 mg. per 100 ml. during winter and 11.3 mg. during summer. Holstein milk contained more cholesterol in summer than winter, but the reverse was true for Jerseys. These seasonal differences were barely significant. The data from Guernsey cows were less complete and a similar comparison was not made. The milk from Holsteins contained less cholesterol than the other breeds during the winter, but differences between breeds were not significant for summer milk because of comparatively large variations between cows of the same breed during this season.

Significant correlations were found between fat and cholesterol contents during both winter and summer. R. K. Waugh

98. Uber den Nachweis der Kurzerhitzung der Milch mit Hilfe der Phosphatasereaktion. (On the phosphatase test for checking of short time heating.) English summary. G. Schwarz and O. Fischer. Die Milchwissenschaft, **3,** 2: 41–45. 1948.

Comparisons between the Scharer and the Kay and Graham phosphatase tests showed both tests to be equally sensitive. However, the difficulty in obtaining the rather expensive phosphotungstic acid and molybdate required in the latter test induced the authors to use the Scharer method. The Scharer test was modified in that the lead acetate was replaced by $ZnSO_4$; borax by Na_2CO_3 and $NaHCO_3$, and by the addition of N/4 NaOH to adjust the reaction to pH 9.3. The modified Scharer test required the following reagents: (a) 1 g. disodium phenylphosphate (phenol free) together with 1 g. Na_2CO_3 and 9 g. $NaHCO_3$, dissolved in water to make 1l. Prepare fresh daily. (b) 10% $ZnSO_4$; (c) N/4 NaOH; (d) 10 mg. 2,6-dibromoquinonchlorimid in 10 ml. of 96% alcohol. Prepare fresh daily and keep away from light.

Procedure: To 0.5 ml. of milk add 10 ml. of buffer (a). Mix and hold for 2 hr. at 37° C. Add 1 ml. of 10% $ZnSO_4$. Mix well and add 1.5 ml. N/4 NaOH and filter through hardened filter paper (Schleicher and Schull 605, extra hard). Add to the clear filtrate 0.5 ml. N/4 NaOH and 10 drops dibromoquinonchlorimid reagent. Hold for 15 minutes and determine extinction coefficient, using filter 561. By omitting the use of $ZnSO_4$ and by increasing the holding time at 37° C. from 2 to from 4 to 6 hr., positive tests were obtained (a) if milk was heated for 30 minutes at 61° C. instead of at 63° C., (b) if milk was heated at 62° for 20 minutes instead of 30 minutes, (c) if milk was heated for short time holding at 69° instead of 71° C., (d) if 0.2% raw milk was added to properly pasteurized milk.

In order to prevent bacterial action during the longer holding time, p-chlorobenzoic acid was added to reagent (a) at the rate of 5 g. per liter. Although a Pulfrich photometer was used to make the readings, the observations also can be made with the naked eye with a little practice.

I. Peters

99. The use of ascorbic acid in controlling oxidized flavor in milk. B. WEINSTEIN, M. LOWENSTEIN and H. C. OLSON, Okla. Agr. Expt. Sta., Stillwater. Milk Plant Monthly, **37,** 10: 116–119. Oct., 1948.

The object of this experiment was two-fold, namely: (a) to determine how much ascorbic acid must be added to prevent the development of an oxidized flavor, and (b) how much ascorbic acid must be added to prevent the development of the off flavor in milk which contained various amounts of added copper. All determinations of ascorbic acid were made utilizing the modified Woessner method.

Milk produced in winter showed a greater tendency to become oxidized than that produced in spring. Addition of 35 mg. of ascorbic acid per 1. of milk prevented development of an oxidized flavor in milk held at 45° F. for 72 hr., although identical unfortified samples became oxidized. In no case did 35 mg. of ascorbic acid prevent oxidized flavor in samples containing 0.25 p.p.m. or more of added copper. In those samples containing 0.25 p.p.m. of added copper, 50 mg. of ascorbic acid was necessary to prevent the defect and in severe cases, such as samples containing 1 p.p.m. of added copper, as much as 150 mg. of ascorbic acid was required. In all cases milk which became oxidized possessed a lower ascorbic acid content at the end of the storage period than those samples which did not become oxidized.

J. A. Meiser, Jr.

100. Lactic acid polymers as constituents of synthetic resins and coatings. P. D. WATSON, Agr. Research Admin., U.S.D.A., Washington, D. C. Ind. Eng. Chem., **40,** 8: 1393–1397. 1948.

Lactic acid is produced by fermentation of the carbohydrates present in corn sugar, molasses and whey. Although cheese and casein whey are a potential source for 400,000,000 lb. of lactic acid, only 6,000,000 lb. are produced annually from all sources. The relatively high price of the purified acid has retarded its greater use. About 400,000 lb. now are used in the plastics industry. This paper describes modified lactic acid condensation polymers which may be of interest to the coatings industry. The most useful of these products appears to be a modified polylactylic acid-fatty oil polymer, from which tough, water-resistant coatings may be formulated. Another class of resins is the metal polylactyl lactates derived almost entirely from lactic acid; these may be used for protective and decorative coatings.

B. H. Webb

Also see abs. no. 82, 83, 107.

DAIRY ENGINEERING

A. W. FARRELL, SECTION EDITOR

101. Vacuum treatment of milk powder. H. SHIPSTEAD. (Assigned to Borden Co.) U. S. Patent 2,453,277. 3 claims. Nov. 9, 1948. Official Gaz. U. S. Pat. Office, **616,** 2: 444. 1948

Milk is spray dried in the conventional manner except that the moisture content is higher than that desired in the final product. The hot powder is conveyed without loss of heat to a vacuum chamber where a portion of the moisture in the powder is flashed off and the product is cooled.

R. Whitaker

102. Method for the evaporation and concentration of liquids. G. G. ZAHM. (Assigned to Hurd Corp.) U. S. Patent 2,450,774. 3 claims. Oct. 5, 1948. Official Gaz. U. S. Pat. Office, **615,** 1: 209. 1948.

Liquid food products such as milk are passed in a film over a heat exchanger under vacuum. The vapors are condensed under vacuum and the flavor containing constituents in concentrated form may be collected and returned to the concentrated solids if desired. The process is conducted rapidly at a high vacuum and relatively little heated flavor is produced.

R. Whitaker

Also see abs. no. 116, 122.

DAIRY PLANT MANAGEMENT AND ECONOMICS

103. The future of milk consumption. A review of the main factors influencing milk consumption. L. SPENCER, Cornell Univ., Ithaca, N. Y. Milk Plant Monthly, **37,** 10: 124–130. Oct., 1948.

Those factors of greatest importance in influencing future milk consumption in the U. S. are: (a) population, (b) convenience, service, quality and availability of milk as compared to other foods. The author concludes that the general outlook of milk consumption is favorable, although there is a definite need for improving the attitude of the consumer towards the dairy industry. This he reasons can be done only by an increased effort to inform the public as to costs, prices and profits encountered by the industry, as well as to the nutritive value of the product.

J. A. Meiser, Jr.

104. How the Boston formula works. W. C. WELDON, H. P. Hood and Sons, Boston, Mass. Am. Milk Rev., **10,** 11: 32, 34, 78, 79. Nov., 1948.

A new formula for determining the price which farmers receive for class 1 or fluid milk was put into effect in the Federal order markets of Boston, Fall River and Lowell-Lawrence on April 1, 1948. The formula is based upon an average of three separate indices, (a) wholesale commodity price index for the entire United States, as prepared by the Bureau of Labor Statistics, (b) department store sales index for New England, published by Federal Reserve Bank, and (c) farm labor and feed cost index prepared by the Federal Market Administrator. The average index is applied to the base price which existed in 1925–29. Price changes occur at 22-cent intervals when called for by the formula. Two additional price stipulations were super-imposed on the above formula price. A seasonal control factor provides for an increase of 44 cents per 100 lb. in the fourth calendar quarter and 44 cents decrease in the second calendar quarter. A supply-demand control factor provides for a decrease of 44 cents per 100 lb. if a surplus greater than 41% exists for the previous 12 months, and an increase of 44 cents if the surplus is less than 33%.

The older method of arriving at milk prices by making use of market quotations for butter and skim milk powder was unsatisfactory, and price changes subject to public hearings likewise proved unsatisfactory. Acceptance of the new formula has been favorable among all groups, but the real test will come when price reductions are called for. D. J. Hankinson

105. Laboratory control of finished dairy products. K. G. WECKEL, Univ. of Wis., Madison. Milk Plant Monthly, **37,** 9: 34–36. Sept., 1948.

The author divides the laboratory control of dairy products into four phases: (a) chemical control, (b) bacteriological control, (c) control of physical properties, and (d) control of organoleptical properties. A brief discussion of methods used to effect these controls is presented. J. A. Meiser, Jr.

106. Ten tips to thrift. A. C. KIECHLIN, Pompton Lakes, N. J. Ice Cream Rev., **32,** 5: 37, 72–74. Dec., 1948.

The author offers 10 helpful suggestions for keeping the tax expense at a minimum. The suggestions offered are as follows: (a) Deduct adequate depreciation to cover normal wear and tear on business property, machinery, non-mechanical equipment trucks, fixtures, etc. (b) Trade-in allowances on equipment when properly handled may result in an income tax reduction. Whenever the trade-in allowance of equipment is less than the unrecovered book value of the equipment, a tax saving will result by selling the equipment for cash and recording the loss on the books. Such losses then may be deducted on the income tax return. (c) Repair and maintenance costs should be considered as expense items which are deductible from income. Such items should not be considered as increasing the value of the assets of the business in which case they will increase the amount of tax to be paid. (d) Bad debts, both personal and business, should be written off and deducted. (e) Non-business expenses which include any items paid during the taxable year in connection with the earning of taxable income should be recorded and deducted. (f) Inventories should be carefully recorded and figured at cost or market value whichever is the lower. (g) Since deductions overlooked one year are not deductible the following year, it is important that the tax return be set up in rough form before the books for the current year are closed. The return then can be examined to make certain no transactions which might reduce the income tax have been overlooked. (h) Transactions throughout the year should be followed closely to make certain they are recorded to conform with those regulations which will give the maximum relief from taxes. (i) Record promptly and accurately all transactions. Do not trust to the memory to recall details of transactions several weeks or months old. (j) Figure the income tax early enough to make it possible to determine what effect, if any, certain adjustments might have on the tax to be paid. W. J. Caulfield

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

107. Comparison of methods for the determination of carotene. J. V. DERBY, JR., and J. B. DEWITT, U. S. Dept. of Interior, Fish and Wildlife Service. J. Assoc. Offic. Agr. Chemists, **31,** 4: 704–708. Nov., 1948.

Replicate samples of dehydrated alfalfa leaf meal were assayed for carotene content by using the methods of Wall and Kelley (Ind. Eng. Chem., Anal. Ed., *15*: 18. 1943), a modified A.O.A.C. procedure, saponification of the extracts of the modified A.O.A.C. procedure and an alcohol potash digestion method for extraction of pigments. When the extinction coefficients of the carotene solutions were plotted

against wave length, the results obtained by the 4 procedures varied. The curves indicated that the extracts differed in amounts of other carotenoid pigments. The solution obtained by the Wall and Kelley procedure appeared to contain the highest percentage of pure β-carotene, although the total content of carotenoids was lower than that of any of the other methods. The amount of carotene found by the modified A.O.A.C. procedure was 45% greater than that found by the Wall and Kelley method. Differences observed in the results of assays on replicate samples were thought to be due to varying degrees of efficacy of the extraction technics. F. J. Babel

108. Wood yeast as a protein supplement. E. G. Ritzman, Univ. of N. H. Guernsey Breeders' J., **74,** 6: 1025–1026, 1070. Nov., 1948.

Two groups of three Guernsey cows each, were fed a grain mixture containing wood yeast as a protein supplement. Soybean meal was used in the check ration. The grain mixtures contained approximately 20% crude protein, with 234 lb. of wood yeast replacing 250 lb. of soybean meal.

Group I was fed the mixture with wood yeast for 4 weeks and then switched to the mixture with soybean meal for 4 weeks. Group II was fed in the reverse order. The wood yeast appeared to give as good results in milk production as soybean meal. A. R. Porter

Also see abs. no. 111, 124.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

109. Your herd sire—expense or investment? G. E. Gordon, Univ. of Calif. The Jersey Bull., **67,** 22: 1932–1933, 2041–2045. Nov. 25, 1948.

Cost figures from the San Pasqual Breeders Association, Inc., San Diego County, California, show $146.53 annual costs per bull for investment and capital. Current annual costs averaged $222.00 for feed, $81.11 for labor and $7.50 for veterinary service and miscellaneous, or a total annual cost of $457.14 per bull.

A bull with 8 daughters averaging 42 lb. of butterfat more than their dams is estimated to contribute an additional $336.00 per year to the herd income or $1680.00 increased income over a 5-year period. In addition, his bull calves sold for higher prices and his daughters had greater sale value. Bull calves from an unproved bull sold for an average of $168.00. After his proving with 110 lb. of butterfat increase for his daughters over their dams, his sons sold for an average of $455.00 each. The figures given show the need for careful selection of a herd sire as a good investment. A. R. Porter

110. Die Leistungsvererbungen Schweizer Braunviehstiere in der Allgäuer Herdebuchzucht, unter besonderer Berücksichtigung des Fettprozentgehaltes. (The heritable factors of the Brown Swiss bulls in the Allgäu pedigree cattle breeds with special consideration of the percentage of butterfat.) English summary. H. Korprich. Die Milchwissenschaft, **3,** 4: 89–97. 1948.

The records of 13 domestic and imported Brown Swiss bulls were examined for herd improvement. The milk yield and the per cent butterfat of the daughters were compared with that of the respective dams of the domestic Allgäu breed. The results showed that both superior and inferior bulls had been imported from Switzerland. It was concluded that the import of expensive sires was justifiable only in cases where herd improvement was certain. I. Peters

111. Forage crop management for higher yields. C. M. Harrison, Mich. State College, E. Lansing. Hoards Dairyman, **93,** 22: 859. Nov., 1948.

The value of the following mixtures for hay, pasture and green manure was studied: I. Red clover, alsike clover, white clover, timothy and Kentucky blue grass. II. Timothy, smooth bromegrass, perennial rye grass, Kentucky and Canada blue grass. III. Alfalfa, red clover, alsike, white clover and smooth bromegrass. IV. Alfalfa, red clover, alsike clover and white clover. Four blocks of each mixture were left for hay, 4 were pastured continuously by sheep and the remaining 4 were used for first hay and second crop pasture. The three systems of management were carried on over a 3-year period. Mixtures III and IV produced approximately twice as much hay over the 3-year period as did mixtures I and II. Mixture I (predominantly red clover the first year) produced very little more the first year or in total than did mixture II. Mixtures I and II produced no second cutting in 2 of the 3 years and a comparatively small amount only in the first year. Yields under grazing treatment varied in much the same manner as they did when cut for hay. During the 3 years, mixture I produced 9,079 lb. hay and 828 days of grazing; mixture II, 8,213 lb. hay and 774 days; mixture III, 14,703 lb. and 2,058 days; and mixture IV, 11,689 lb. and 2,022 days. It appears that mixtures containing alfalfa were almost twice as productive as the straight grass or grass-red clover mixture when measured as hay or by grazing livestock. J. B. Frye, Jr.

ICE CREAM

C. D. DAHLE, SECTION EDITOR

112. Ice cream mixes as influenced by homogenization of only a part of the mix. W. A. KRIENKE and R. A. HIBBS, Fla. Agr. Expt. Sta., Gainesville. Ice Cream Trade J., **44,** 11: 48, 49, 78, 79. Nov., 1948.

Fractional homogenization of ice cream mixes made from cream (40% fat), condensed skim milk (36% serum solids), sodium alginate and water (180 p.p.m. hardness) proved satisfactory when 54% of the mix containing all the milk products was homogenized. Satisfactory whipping resulted and the body and texture score of the ice cream was only slightly below that of ice cream made from a completely homogenized mix. This practice if used would result in some saving on power and a smaller homogenizer could be used. W. H. Martin

113. Superheating ice cream mixes. W. H. MARTIN, Dairy Husb. Dept., Kansas State College. Ice cream Field, **52,** 4: 65–6. Oct., 1948.

The author reports results obtained on superheating ice cream mixes by injecting live steam into mix made in a vacuum pan. Heating the mix at 190° F. for 10 minutes increased mix viscosity 33%, whereas heating at 210° F. for 10 minutes increased mix viscosity 250% over that of mixes not superheated. The whipping properties of mixes were impaired slightly by superheating. Superheating mixes at 190° F. for 10 min. or at a higher temperature or for a longer time impaired the flavor of the resulting ice cream. The body and texture of ice cream made from superheated mixes containing stabilizer were equal to and often superior to ice cream manufactured from mixes not superheated. Ice cream made from superheated mixes containing no stabilizer was coarse in texture.

Bacterial destruction as a result of superheating was efficient. Counts of 200 to 400 bacteria per ml. were common, and it is claimed that the process can be depended upon to reduce the bacteria count to 10,000 per ml. or less.

W. C. Cole

114. Storing milk solids for ice cream. C. D. DAHLE, W. F. COLLINS, and J. A. MEISER, JR., Dairy Dept. Penn. State College. Ice Cream Field, **52,** 4: 53. Oct., 1948.

The following recommendations are made:

Frozen cream: Use sweet cream with 40% fat or more, pasteurize at 170° F. for 10 minutes or longer, cool and run into new tinned 50 lb. cans. Freeze at −15° F. and store at about −10° F. Avoid copper equipment. Employ suitable crushers or slicers to avoid mess and work of defrosting.

Plastic cream: Precautions for frozen cream apply except that the fat content is about 80%. Use essentially as butter of 93 score.

Concentrated mix: A concentrated mix or a blend of fat and solids-not-fat of the proper ratio can be frozen and stored. Heat to 170° F. for 10 minutes before condensing. Use copper free equipment.

Frozen mix: Pasteurize the whole mix at 170° F. for 20 minutes before freezing; may be stored for 6 months and does not require rehomogenization, but authors advise storing concentrated mix instead.

Frozen condensed milk: Plain condensed skim milk tends to curdle when frozen and held for long periods of time, but the product will disperse in the hot mix if handled properly. They recommend preheating of skim milk at 160 to 170° F. instead of the higher temperatures commonly used; condense to 3:1 or 4:1 and freeze; store at −10 to −15° F. Either plain or superheated condensed skim milk may be frozen and stored, and the product has been useable after 6–12 months in storage. W. C. Cole

115. Fried ice cream. ANONYMOUS. Ice Cream Rev., **32,** 5: 40. Dec., 1948.

A new novelty item recently introduced in the ice cream field is "Frigid Frys" or fried ice cream. In making this novelty a portion of ice cream of any desired shape is covered with a specially prepared dough and fried in deep fat for approximately 1 minute. This will cook the dough to a light brown color without melting the ice cream. Frigid Frys require no special dishes for serving and are well adapted for the carry out trade. They are said to have all the sales appeal of pie a la mode. It is anticipated that exclusive dealer territory will be established for distribution of this new novelty item.

W. J. Caulfield

116. Wind tunnel eliminates need for big hardening room. ANONYMOUS. Ice Cream Rev., **32,** 4: 74, 76. Nov., 1948.

A compact wind tunnel costing approximately $3,000 has been installed in the plant of the Kilgore Eastland Creamery, Eastland, Texas. The tunnel with suitable shelving occupies a space 3 feet wide, 6 feet high and 8.5 feet long. It is capable of hardening the output of an 80-gallon per hr. continuous freezer in a continuous movement process.

When a container of ice cream from the freezer

is placed on a shelf on the tunnel, it is used to push ahead the container in front of it. By the time the ice cream has travelled the length of the tunnel, it is thoroughly hardened and may be stored in the hardening room or loaded directly onto trucks.

Advantages claimed for the comparatively low-cost hardening tunnel are smoother textured ice cream and less need for maintaining a large stock of ice cream on hand. W. J. Caulfield

117. Refrigerating with dry ice. G. B. Armstrong, Mathieson Chemical Corp. Ice Cream Field, **52,** 2: 31, 34, 35. Aug., 1948.

Dry ice now is being used in large trucks for wholesale delivery and small trucks for door-to-door sales, as well as in ice cream cabinets, in retail stores and to refrigerate take home packages of ice cream. In wholesale delivery trucks, bunkers are installed so they will be accessible when the truck is fully loaded. Air circulation may be helped by installing blowers. For economical use of dry ice refrigeration of trucks, (a) adequate bunker capacity, (b) use of economical design of truck body, (c) efficient insulation—at least 6 inches of standard insulation and (d) proper hardening of ice cream before loading truck are stressed. A well-constructed ice cream truck body with 3 compartments (400 gal. capacity) should not exceed an average daily requirement of 50 lb. of dry ice. A 2-compartment dry ice cabinet in a room at 70° F. requires about 11 lb. per day. An ice cream packing chart for dry ice accompanies the article.

W. C. Cole

118. Abbotts solves its truck moisture problems. E. W. Holgren, Abbotts Dairies. Ice Cream Field, **52,** 2: 32–33. Aug., 1948.

For somewhat over 20 years, Abbotts Dairies Fleet Maintenance Dept. has had in affect a checking system to reduce to a minimum the accumulation of moisture in truck bodies. This check includes: (a) Weighing of the truck annually to determine any increase in gross weight of empty trucks compared with these weights when the trucks were new; the cause of any increase is determined. (b) A regular inspection is made of the refrigerated plates. (c) The electrical energy required to operate the compressors is measured. (d) All refrigerated units are defrosted once a month.

An expanded polystyrene is used for insulation, with ends and sides 6 in. thick and floor and roof 8 in. thick. A water asphalt emulsion is used on the joints and on the outside of the insulation just under the metal covering.

The delivery trucks are equipped with mechanical refrigeration compressors of 1.5 H.P. capacity connected to 4 eutectic plates, while the transport trucks are refrigerated with dry ice. W. C. Cole

119. Ice cream preference tests. P. S. Lucas, Mich. Agr. Expt. Sta., E. Lansing. Am. Milk Rev., **10,** 12: 56–58. Dec., 1948.

Pure vs. imitation vanilla and strawberry flavors were rated in order of preference by the general public. Five combinations of vanilla flavoring were rated by 44 persons as follows: first—pure vanilla; second—imitation vanilla; third—pure vanilla reinforced with vanillin; fourth—pure vanilla reinforced with coumarin and fifth—vanilla fortifier only. Five combinations of strawberry flavoring were rated by a group of 15 experienced scorers as follows: first—true fruit strawberry extract; second—true fruit strawberry concentrate; third—strawberry flavor, single strength, reinforced; fourth—imitation strawberry flavor and fifth—special imitation strawberry. The above 5 strawberry flavor combinations were rated by 23 inexperienced scorers in the same order as the experienced scorers except the second and third ratings were reversed. The above strawberry flavoring materials were added to 5 gal. ice cream mix plus 3 qt. strawberries. It was concluded from this study that consumers prefer pure flavoring materials in ice cream. D. J. Hankinson

120. Slide rule to greater dealer profits. T. E. Heindenreich, Jr. Ice Cream Trade J., **44,** 11: 33, 71, 72. Nov., 1948.

A slide rule to be used in the determination of the exact cost of any fountain item has been developed by the General Ice Cream Co. of Schenectady, N. Y. "The working portion of the rule is a slide which can be set at the cost per gallon of ice cream. Four sets of figures come into view showing the exact cost of 5 different scoop sizes, 4 weights of pints, 5 weights of quarts and the factory filled pints. All figures are based on a 78 oz. gallon." W. H. Martin

121. Combined spoon and lid for containers. R. C. Wilson. U. S. Patent 2,453,393. 2 claims. Nov. 9, 1948. Official Gaz. U. S. Pat. Office, **616,** 2: 474. 1948.

The disk-shaped lid of a paper cup, suitable for ice cream, is scored in such a manner that a section of the lid may be easily detached and folded to form a spoon-like member.

R. Whitaker

Also see abs. no. 87, 88.

MILK

P. H. TRACY, SECTION EDITOR

122. Die Milchannahme vom Fahrzeug bis zum Annahmebehalter. (Reception of milk.) English summary. W. SCHULZ. Die Milchwissenschaft, **3,** 2: 29–36. 1948.

Milk should be loaded into trucks in a manner permitting quick and easy unloading. Loading and unloading from the side is preferable. A mechanical can carrier with moving chains is best and for easy unloading should not be higher than the lowest loaded truck. Cans should pass through rinse, defroster (if necessary) and cover loosener and should be examined on their way to the dump tank. For quick, easy dumping of milk, a suitable can dumper should be installed, requiring a minimum of physical labor, leaving the cans in an inverted position on their way to the can washer. I. Peters

123. Observations on rough or "spotty" homogenized milk. G. M. TROUT and J. R. BRUNNER, Mich. State College, E. Lansing. Milk Plant Monthly, **37,** 10: 92–94. Oct., 1948.

The rough granular substance adhering to the inner surface of a partially emptied homogenized milk bottle was found by microscopic examination to be a fatty material that was stained readily by Sudan III. Routine line checks of the processing operation proved that non-homogenized milk was contaminating the homogenized product in the bottle filler reservoirs. Either ceasing to bottle cream prior to bottling homogenized milk or a thorough clean-up between operations eliminated this defect. J. A. Meiser, Jr.

124. Benzene hexachloride flavored milk. H. G. LINDQUIST and R. W. DONALDSON, Univ. of Mass., Amherst. J. Milk and Food Technol., **11,** 6: 325–326. Nov.–Dec., 1948.

Potatoes grown in fields treated with benzene hexachloride, or 666 to destroy wire worms, were rendered unfit for human consumption. A utilization of these potatoes as cattle feed prompted the authors to study the effects on the flavor of milk from cows fed these products. An off-flavor was observed in the potatoes and in the milk. However, the flavor varies in intensity and only a keen sense of taste will detect it. No specific chemical test was satisfactory to detect the presence of this compound in milk. The investigators are of the opinion that the presence of small amounts of benzene hexachloride in potatoes or in milk obtained from cows fed the potatoes is harmless to humans. H. H. Weiser

125. Paper bottle top. J. P. JONES. (Assigned to Dairy Specialties, Inc.) U. S. Patent 2,453,-133. 2 claims. Nov. 9, 1948. Official Gaz. U. S. Pat. Office, **616,** 2: 408. 1948.

Milk may be poured easily from a paper bottle by inserting this device which consists of a gradually pointed tube, ending in a discharge spout and provided with a projection to determine extent of insertion. R. Whitaker

126. Milk delivery tube. J. A. HOPWOOD. (Assigned to Monitor Process Corp.) U. S. Patent 2,449,229. 2 claims. Sept. 14, 1948. Official Gaz. U. S. Pat. Office, **614,** 2: 455. 1948.

A tube for draining milk from milk cans is described; it may be detached easily for cleaning. R. Whitaker

127. The reseparation method for increasing the viscosity of cream. A. C. SMITH and F. J. DOAN, Penn. Agr. Expt. Sta., State College. Milk Plant Monthly, **37,** 10: 84–88. Oct., 1948.

Using a variable speed separator and maintaining accurate temperature controls, the authors found that the increase in viscosity obtained by the reseparation method was not due to centrifugal action, but was the result of a controlled heating and cooling treatment. This treatment, they reasoned, was nothing more than a variation of Hening and Dahlberg's rebodying process. The reseparation method offered several disadvantages in that it enhanced cream plug formation and serum separation in aged cream and necessitated the use of an extra piece of equipment. J. A. Meiser, Jr.

128. Cream defects—their causes and preventions. LYNN R. GLAZIER, Pfaudler Co., Rochester, N. Y. Am. Milk Rev., **10,** 12: 64–65. Dec., 1948.

The 10 defects described are cream plug, feathering, foaming, high acidity, lipase activation, off-flavors, oiling-off, poor whipping ability, poor viscosity and serum separation. Causes and preventions are listed in tabular form. D. J. Hankinson

129. Milk fat in milk chocolate. L. W. FERRIS, Food and Drug Admin., Federal Security Agency, Buffalo, N. Y. J. Assoc. Offic. Agr. Chemists, **31,** 4: 728–731. Nov., 1948.

Results are presented which show that straight ether extraction of milk chocolate leaves an appreciable amount of milk fat unextracted. A method is presented which effects a more complete extraction of the fat. The method consists of extraction with ether, followed by the addition

of 1,4-dioxane and heating and final extraction with ether. In one instance slightly more than 1% of additional milk fat was obtained by the proposed method. F. J. Babel

130. Milk vending—a new horizon. E. J. Newcomer, City Milk Co., Inc. Am. Milk Rev.,**10,** 11: 40, 42. Nov., 1948.

Vending machines have not been used widely for dispensing milk. Health regulations and the wide variety of bottles used for milk have discouraged the design of automatic milk vending machines. However, automatic equipment now is available to dispense all types of containers, and some machines will dispense both chocolate and plain milk. When two products are delivered by the same machine, it is possible to serve as many as 168 persons in 15 minutes. Automatic vending equipment is compared with manually operated equipment, which served only 35 units in 15 minutes. Automatic vending equipment is considered most adaptable to factory service and apartment houses. Machines must be capable of accepting varying amounts of money, because the price of milk varies widely in different areas. Either the 1/3 qt. or 10-oz. bottle is recommended. It is pointed out that consumers readily choose milk instead of other beverages where both milk and beverage vending machines are in use. D. J. Hankinson

131. Concentrated apple juice and milk modifier. H. St. Clair. (Assigned to Ridgewood, Inc.) U. S. Patent 2,450,456. 10 claims. Oct. 5, 1948. Official Gaz. U. S. Pat. Office, **615,** 1: 133. 1948.

Milk for feeding infants and children is made more digestible and nutritious by blending with concentrated apple juice having a pH of 4.5 to 5.5 and a density of 68 to 75° Brix.

R. Whitaker

Also see abs. no. 89, 93, 97, 98, 99, 103, 104, 133.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

132. Die Regulierung der Laktation. (**The regulation of the lactation.**) English summary. E. Fauvet. Die Milchwissenschaft, **3,** 3: 61–65. 1948.

Lactation is a nutritive process found in mammals as well as in some lower classes of animals. In the lower classes of animals, some males and gonad-less individuals are capable of nursing the young. During the process of evolution, lactation has become an economic and purely maternal passive performance.

In the higher mammals the rudimentary mammary glands develop during pregnancy and start functioning autonomically after the disappearance of progesteron. The pituitary gland takes no active part in this process at first, since the hypothetically assumed milk-forming hormone does not exist. Only through the stimulatory act of sucking is the pituitary gland caused to produce a histotrope hormone, the latter maintaining and renewing continuously the parenchyma of the lactative glands. By means of this change, the mammary gland becomes independent of its follicle hormone and becomes subordinate to the pituitary controlling growth factor. I. Peters

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

133. The fortification of milk with vitamins A and D. Council on Foods and Nutrition. J. Am. Med. Assoc., **138,** 1: 23. Sept. 4, 1948.

The Council long has approved fortification of milk with vitamin D, but it has stated the opinion that fortification of milk as a general purpose food with vitamin is not in the interest of public health. There is no objection to the presence of viamin A when the preparation used for fortifying with vitamin D contains vitamin A in natural association. Fish liver concentrates often contain large amounts of vitamin A, and milk fortified with these concentrates has been accepted and a statement of vitamin A content has been permitted on the label.

The Council believes that, even though the procedure apparently is harmless, evidence is lacking that fortification with vitamin A is in the interest of public health and does not constitute reason for the acceptance of advertising that suggests special nutritional advantages. Since milk is a good source of vitamin A, additional fortification with vitamin A may add to the cost of the milk. D. P. Glick

SANITATION AND CLEANSING

K G. WECKEL, SECTION EDITOR

134. Wetting Agents. Some facts about the nature, uses and economic importance of wetting agents. R. H. Smith, E. I. Du Pont de Nemours & Co., Chicago, Ill. Milk Plant Monthly, **37,** 9: 80–81. Sept., 1948.

Wetting agents may be classified according to: (a) the source of the raw material used in the manufacture of the compound, (b) the type ion produced when the compound is dissolved in water, and (c) the physical properties of the compound.

The vast majority of wetting agents belong to the anionic group, being produced from non-fat raw materials, and should possess one or more of the following properties: (a) sequestering action, (b) power of penetration, (c) power of suspension, (d) power of dispersion, (e) emulsifying ability, (f) detergency, and (g) ability to lower surface tension.

Considering the above properties, all detergents may be called wetting agents. However, not all wetting agents can be classified as detergents.

J. A. Meiser, Jr.

135. Let's look at brushes. NORMAN MYRICK. Am. Milk Rev., **10,** 12: 34–35. Dec., 1948.

Since 20 to 25% of the labor in a milk plant is expended for cleaning, a knowledge of brushes is important. The diameter of a brush for the inside of sanitary pipe should be greater than the diameter of the pipe, but not so much greater that matting of the brushes occurs and swabbing action instead of brushing action takes place. The bristle should bow somewhat, allowing the end to do the cleaning. Separator discs are cleaned between 2 counter-revolving brushes. Solution-fed brushes are useful for cleaning large surfaces. Nylon bristles, although more expensive than animal fibre bristle brushes, are more durable. Furthermore, nylon bristles are available in various diameters and do not become water-soaked. Motor driven brushes also are useful. Cleaning solution temperature of 115° F. is suggested.

D. J. Hankinson

136. The influence of pH on the efficiency of hypochlorite solutions in sterilizing metal surfaces. C. M. COUSINS and J. WOLF, Milton Antiseptic Ltd., and Deosan Ltd., John Milton House, Brewery Rd., London, N. 7. Proc. Soc. Applied Bact., **1946,** 1: 15–19. 1946.

Experiments were conducted with a culture of *Staph. aureus* and 2 cultures of thermoduric micrococci which were suspended in 10% skim milk, then inoculated on metal trays and dried. The highest destruction of the bacteria by hypochlorites containing 25, 50, 100 and 200 p.p.m. available chlorine were at the alkaline pH values of 9.5, 10, 10.7 and 11, respectively. Spores of *B. subtilis,* however, were destroyed by hypochlorites (50 p.p.m.) more quickly at pH 7 than at higher pH values. This difference in activity for hypochlorites on dried vegetative cells and dried spores was believed to be caused by formation of a chloroprotein in the case of the vegetative cells which have a cell wall of protein nature exposed to the action of dissociated hypochlorite. The refractile walls of spores were considered not to be of this protein nature, their destruction being dependent on the concentration of undissociated hypochlorite present.

M. L. Speck

137. Sterilization of dishes and utensils in eating establishments. G. R. WEBER, Milk and Food Sanitation Lab., U. S. Public Health Service, Cincinnati, Ohio. J. Milk and Food Technol., **11,** 6: 327–333, 351. Nov.–Dec., 1948.

Sanitization of dishes and utensils can be accomplished by cleaning all utensils with an acceptable detergent and rinsed, with hot water at 170° F. for at least 2 min., with chlorine, or quaternary ammonium compounds. Since the latter compounds are not used widely, the author believes that they should be tested carefully before being put into practical use. The type, number of organisms and composition of the water used are important factors in the sanitization of eating utensils.

H. H. Weiser

138. Good housekeeping, an essential of modern dairy plant operation. W. M. ROBERTS, Dairy Manufacturing Dept., North Carolina. Milk Plant Monthly, **37,** 10: 102–104. Oct., 1948.

Good housekeeping in the dairy plant insures a high quality product that is more economical to produce. It provides sound advertising and prevents accidents in the plant that may lower the morale of the worker.

Certain questions that may be used in evaluating your plant from the good housekeeping standpoint are: (a) Are the outside surroundings of the plant neat and attractive? (b) Does the appearance of the manager's office set the standard for the plant? (c) Are the laboratories spotless and well arranged? (d) Are the processing rooms clean and devoid of leaky fittings? (e) Are adequate storage facilities provided, and are they kept clean? (f) Are locker rooms and toilets maintained in an orderly and sanitary manner? (g) Does the engine room meet the standards of the remainder of the plant?

It is the responsibility of the plant owner or manager to insure good housekeeping in the plant, and it is his duty to direct, instruct and provide facilities for maintaining good housekeeping in the plant.

J. A. Meiser, Jr.

139. Clip for cleaner milk. N. N. ALLEN, Univ. of Wis., Madison. Hoards Dairyman, **93,** 22: 851. Nov., 1948.

In this study every other cow in the milking line had the udder, flanks, thighs and tail clipped in the manner generally recommended. Alternate cows were unclipped. The cows were milked by hand and machine during the experiment. Samples for bacteria and sediment tests were taken before the milk was strained. The

bacteria count of the milk produced by the clipped cows definitely was lower than that of the unclipped cows when the cows were hand milked. When the cows were machine milked, the averages were in favor of the clipped group. Clipping did not reduce the sediment in the milk enough to change the grade. However, it is pointed out that the sediment test is not a very exact one and will measure only marked differences. J. B. Frye, Jr.

140. Types of material in accumulated dust and fine debris. T. J. CLAYDON, Kansas Agr. Expt. Sta., Manhattan. Am. Milk Rev., **10,** 11: 24, 76, 77. Nov., 1948.

The dust and debris accumulations on ledges in creameries, cream stations and farms are shown to be closely related to the type of sediment found in empty shipping cans and in sediment tests of cream. Microscopic examinations of 80 samples of fine material collected at 3 creameries, 40 cream stations and 6 farms were made. The material found was classified into one of ten types. The occurrence of the various types of material in the 80 samples was as follows: vegetable parts, 97.5%; sand, 92.5%; coal dust, 82.5%; fibers, 76.2%; rodent-like hairs, 53.7%; other hairs, 67.5%; feather parts, 50.0%; insect parts, 38.7%; metal parts, 33.7%. Material not classified in one of the above types was listed as unidentified.

Rodent-like hairs occurred most frequently in the farm samples. Insect parts occurred least in cream station samples. Coal dust and fibers occurred least in farm samples. The importance of controlling airborne contamination of cream and cream cans is indicated. D. J. Hankinson

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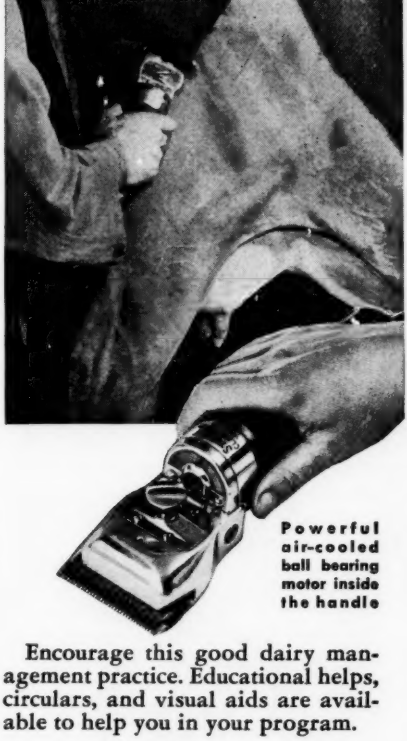

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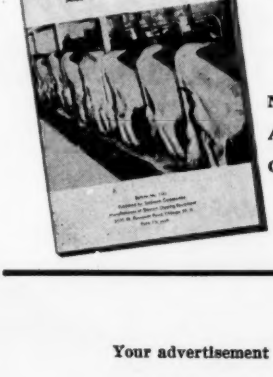

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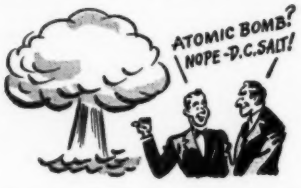

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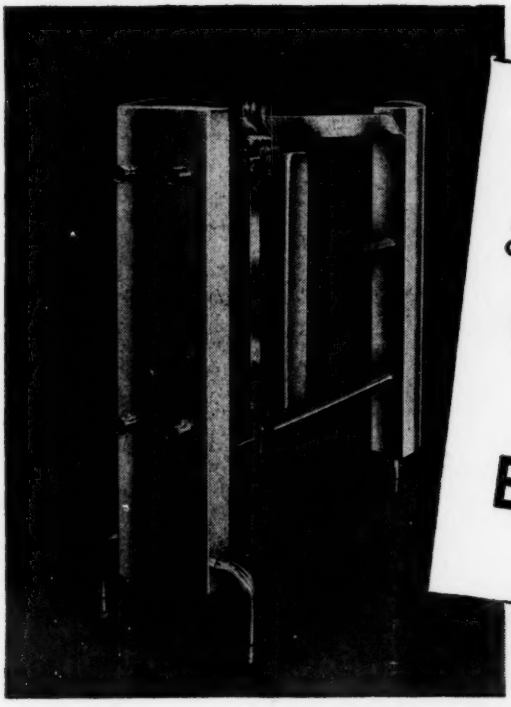

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